The Rapid Detection of MRSA

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Abstract

The control of meticillin-resistant *Staphylococcus aureus* (MRSA) is a global healthcare priority. Screening patients for MRSA carriage occupies a central position in this control. Conventional culture methods for MRSA screening take 2-3 days to produce a positive result. Polymerase chain reaction (PCR) based systems can detect MRSA within one day ('rapid screening') though these tests are more costly than culture tests. It is hypothesised that rapid detection of MRSA carriers will lead to faster implementation of control procedures, reducing the transmission of MRSA.

The first study in this thesis validates a rapid method, IDI-MRSA™ for use on pooled and non-nasal specimens. The second paper is a controlled trial of the IDI-MRSA™ test. It investigated whether rapid MRSA screening leads to a reduction in MRSA acquisition and was set on ten wards of a London teaching hospital. The main outcome was the MRSA acquisition rate (proportion of patients negative for MRSA who became MRSA positive). Secondary outcomes included test characteristics and measures of resource use. The intervention was PCR screening for MRSA compared with conventional culture. 6888 (82.3%) patients had full data. The overall MRSA carriage rate on admission was 6.7%. Rapid tests led to a reduction in median reporting time from admission (46 to 22 hours, $P<0.001$) and reduced the number of inappropriate pre-emptive isolation days between the two arms (399 v 277, $P<0.001$). 108 (3.2%) patients in the control arm and 99 (2.8%) in the intervention arm acquired MRSA. When confounding factors were taken into account the adjusted odds ratio was 0.91 (95% confidence interval 0.61 to 1.234). Rates of MRSA transmission, wound infection, and bacteraemia were not statistically different between the two arms. On these data it is unlikely that the increased costs of rapid tests can be justified compared with alternative control measures against MRSA.

Acknowledgements

I am very grateful to the staff and patients of Guy's and St. Thomas' NHS Foundation Trust who made these studies possible. I am indebted to Professor Gary L French for his support and advice during my time as a student and beyond. I would also like to acknowledge Reverend Dr John Philpott-Howard for his guidance. Finally, I am hugely thankful to my Mum, Dad and Gavin for all their support.

Contribution to the studies & the thesis

I was the primary researcher for both studies. For the pooling study, I identified, collected and analysed the specimens for IDI-MRSA™ testing. Two of my co-authors helped with the IDI-MRSA™ analysis of the specimens towards the end of that study. I wrote the research article and made the necessary amendments as per my co-authors' suggestions. I set up and ran the randomised, controlled clinical trial. I designed the study and wrote the ethics application with input of the two senior co-authors. I ensured that we had MHRA approval. I taught the ward nursing staff, the laboratory staff and the study staff about the theory of the study and their roles in the study. I ensured that we took part in QC testing and I wrote the laboratory standard operating procedure, which was externally reviewed as part of the laboratory's Clinical Pathology Accreditation inspection. I organised and helped to set up the tests for the study on the laboratory information management system. With three of the research staff, I collected the study data, processed the specimens and participated in the on call rota to ensure the processing of specimens by IDI-MRSA™ on bank holidays and weekends, and out of hours on weekdays. With two co-authors, I set up the study database. I oversaw the cleaning of the data and I cleaned the collected data with two of my co-authors. I was responsible for the validity of the data and I am the guarantor of that data. With two co-authors I did the statistical analysis, which I provided the data for, and I wrote the published research article.

Contents

Chapter 1: General Introduction

1.1 *Staphylococcus aureus* and meticillin resistance

Staphylococcus aureus is a Gram-positive bacterium that colonises mucosal and skin surfaces asymptomatically in about 30% of normal people. However, colonising *S. aureus* may gain entry to tissues through skin breaches and can then cause invasive disease. *S. aureus* is a potentially virulent organism than can infect all organs of the body at all ages. It is the most common cause of infection of skin and soft tissue, wounds (including surgical wounds), bones and joints. It is also one of the commonest causes of bacteraemia and such infections may be fatal.

Patients are termed carriers of *S. aureus* (or meticillin-resistant *S. aureus*, MRSA) if the organisms are isolated from normal carriage sites such as the anterior nares, throat, perineum, groin or axilla. Colonised patients have been defined as those without clinical symptoms who harbour MRSA at non-carriage sites (Garner JS *et al.*,1988); however, for the purposes of this study, the distinction between carriers and colonised patients will not be made. Approximately one third of people are persistent carriers, one third are persistent non-carriers and one third are intermittent carriers (Williams REO, 1963). It is thought that non-antibiotic resistant strains of *S. aureus* are more persistent than resistant strains (Williams REO, 1963). Infected patients are those with active signs of infection.

S. aureus is naturally susceptible to many classes of antimicrobials, including penicillins but it has a great ability to develop resistance to many drug classes simultaneously. Antibiotic resistance facilitates the survival and spread of these organisms in the hospital environment, and multiply resistant strains are often responsible for large and serious outbreaks of nosocomial infection. Since the 1950s, many different resistance problems have been encountered (Shanson DC, 1981). Penicillin resistance due to the production of plasmid-mediated penicillinase appeared in *S. aureus* soon after penicillin was introduced and now most strains are resistant. During the 1950s, multi-drug resistant (MDR) strains of *S. aureus* began to appear and large epidemics of hospital infection with organisms resistant to multiple classes of antibiotics were seen throughout the world. After further outbreaks in the 1970s the incidence of hospital infection with MDR staphylococci gradually declined. The exact reasons for this are unclear, but the decline was associated with the introduction in the

1960s of the penicillinase-stable semisynthetic penicillins, meticillin, nafcillin, oxacillin, cloxacillin and flucloxacillin (which are active against penicillinase-producing staphylococci) and improvements in hospital infection control.

Strains of meticillin-resistant *S. aureus* (MRSA) were noted soon after meticillin was introduced into clinical practice (Jevons MP *et al*., 1963), but they were generally rare until the 1980s despite widespread use of meticillin, cloxacillin, and related drugs. Since the early 1990s MRSA has emerged as a major pathogen of hospital infection in most countries and regions of the world (Brumfitt W & Hamilton-Miller J, 1989). In both the USA and Europe, around 30-50% of hospital blood culture isolates of *S. aureus* are now meticillin resistant (NNIS, 2002; EARSS, 2001), although in the Netherlands and Scandinavia rates are 3% or less.

Meticillin resistance is mediated primarily by the production of an abnormal penicillin binding protein (PBP) called PBP-2a or PBP-2' (Chambers HF, 1997). β-Lactam antibiotics bind to normal bacterial PBPs and inhibit their activity, preventing proper formation of cell wall peptidoglycan and leading to cell death by osmotic lysis. PBP-2a binds poorly with most β-lactams and can fulfil the functions of the so-called essential PBPs 1, 2, and 3. Organisms producing PBP-2a are, thus, resistant to most available β-lactams, including meticillin and the isoxazolyl penicillins. The *mecA* gene encodes the production of PBP-2a. The gene is carried on a mobile genetic element, the Staphylococcus Cassette Chromosome (SCC*mec*). SCC*mec* probably originated in coagulase-negative Staphylococci and integrates site-specifically into the *S. aureus* genome (Zetola N *et al*., 2005; Deurenberg & Stobberingh, 2008). A variety of SCC*mec* types designated I - VI and their variants have been described (Deurenberg & Stobberingh, 2008) and new types continue to emerge. Recent genetic studies suggest that MRSA has repeatedly emerged by meticillin sensitive *S. aureus* (MSSA) strains acquiring *SCCmec* elements at different times in different parts of the world (Hiramatsu K *et al.*, 2001; Enright MC *et al.*, 2002) signifying that there are many clones of MRSA but that some clones have been more successful than others.

1.1.1 The Epidemiology of MRSA in United Kingdom Hospitals

Infections with MRSA are now endemic in United Kingdom (UK) hospitals. Between 1990 and 2004 the proportion of blood culture isolates of *S. aureus* in England & Wales that were meticillin resistant increased from 2% to 40% (Figure 1) (Health Protection Agency, 2007). In 2003, English hospitals reported some 6000 MRSA bacteraemias a year (Figure 2). In the early part of the Millennium, the UK reported one of the highest rates of meticillin resistance amongst bloodstream isolates of *S. aureus* infection in Europe (Gould IM, 2005), second only to Greece (Figure 3). In English surveillance studies *S. aureus* is the most common organism isolated from surgical site infections and 60% of these are MRSA (Health Protection Agency, 2007).

Figure 1: **Percent meticillin resistance in *S. aureus* bacteraemia isolates in England and Wales 1990-2003**

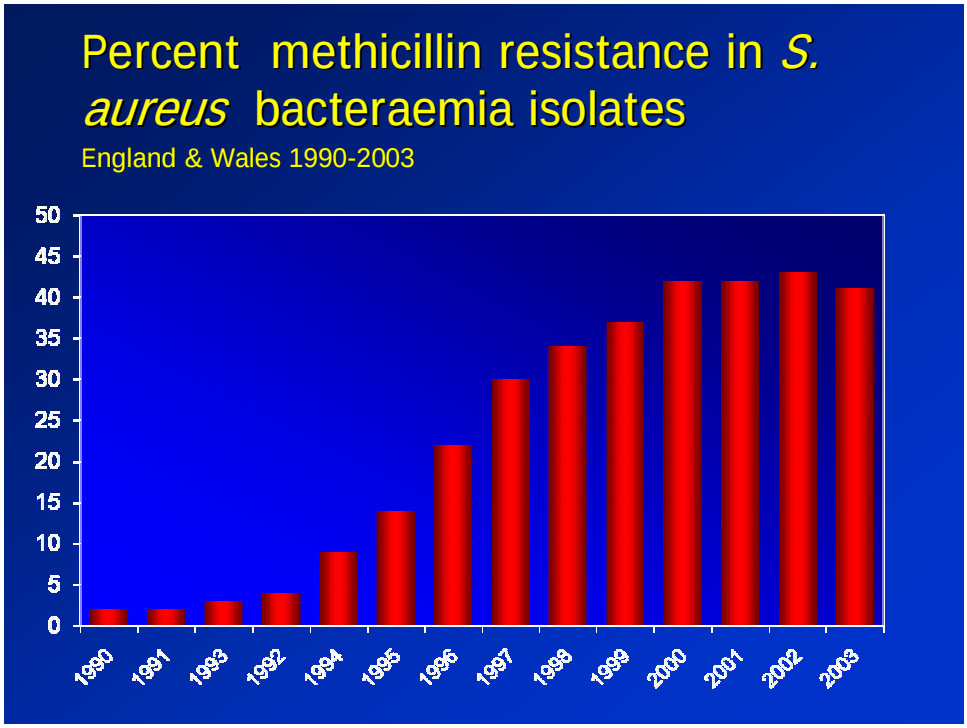


Figure 2: Number of MRSA bacteraemia isolates in England and Wales 1990-2003

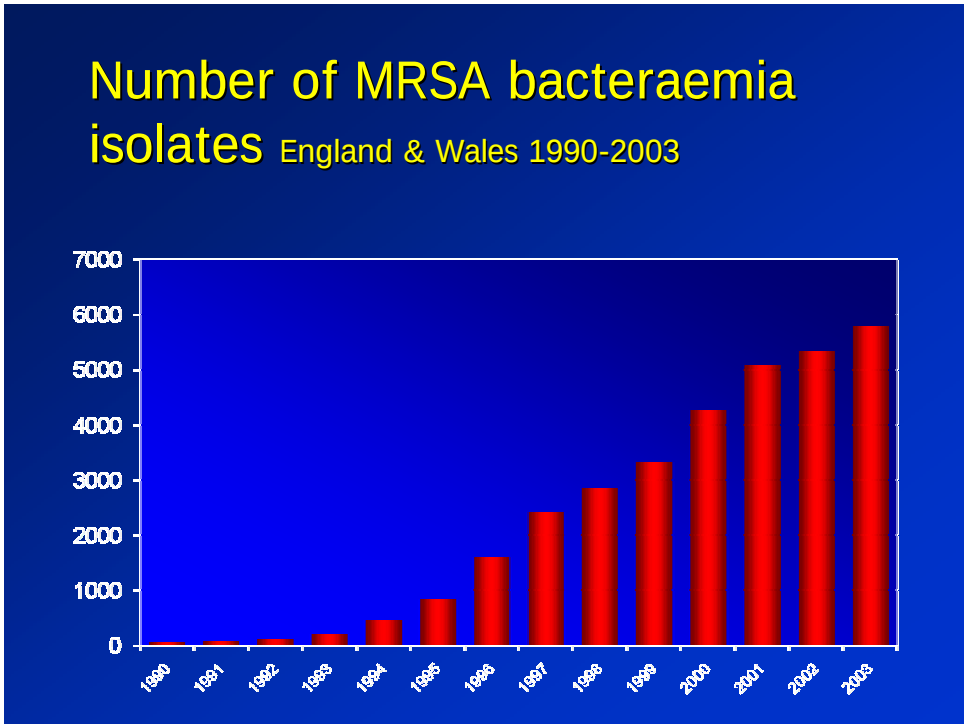
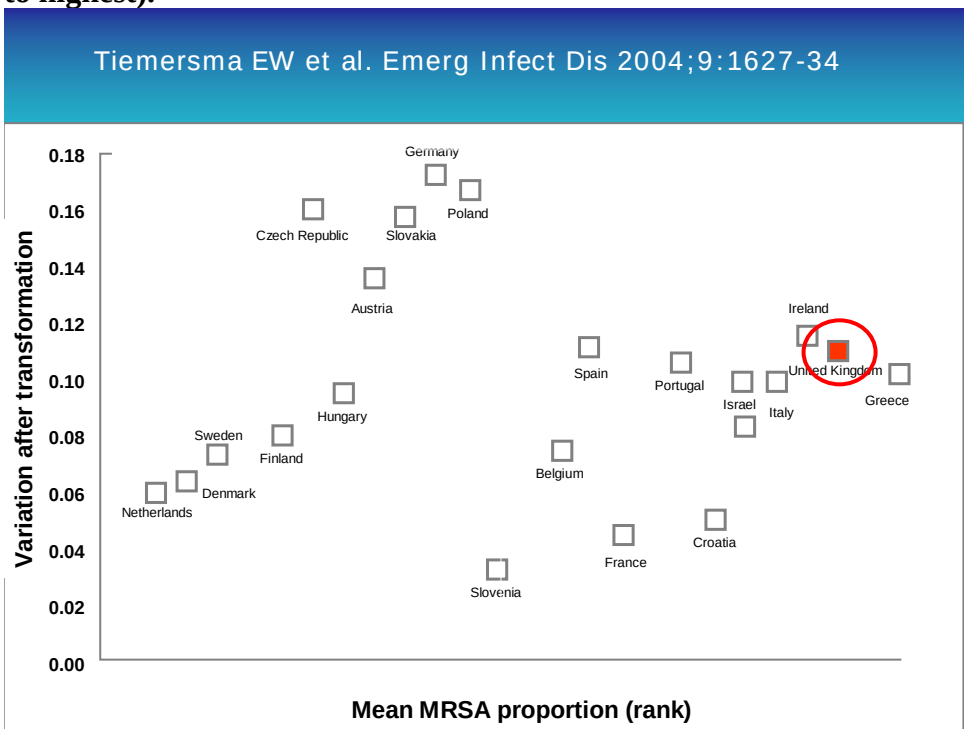


Figure 3: Inter-country comparison with respect to variation between hospitals showing the power-transformed variance being independent of the mean MRSA proportion per country, displayed by ranking of MRSA proportion (from lowest to highest).



After intensive efforts to reduce the incidence of MRSA, a decline in the proportion of *S. aureus* bloodstream isolates with resistance to meticillin was first noted in the UK in 2006 (Table 1) (EARSS Annual Report 2008). By 2010, the UK had demonstrated a year on year reduction with the proportion of isolates due to MRSA falling to 21.6% (95% confidence intervals 20-23) from just over 30% in 2008 (Table 1). This placed the UK 18th of the 28 European countries contributing data (Annual report of the European Antimicrobial. Resistance Surveillance Network (EARS-Net) 2010).

Table 1: Proportion (%) *Staphylococcus aureus* blood culture isolates with methicillin resistance by year 2001 - 2010

2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
44%	44%	43%	44%	44%	42%	36%	31%	28%	22%

There are four primary methods for molecular typing of *Staphylococcus aureus*: pulsed field gel electrophoresis (PFGE), multilocus sequence typing (MLST), *spa* typing and SCC*mec* typing (Mediavilla J R *et al.*, 2012). Almost all MRSA isolates in the UK are healthcare-associated (HA-MRSA) and most are of the HA-MRSA epidemic types EMRSA-15 and EMRSA-16 (Johnson AP *et al.*, 2001), a classification based upon PFGE. MLST is a sequence-based typing method, which analyses fragments of seven housekeeping genes distributed throughout the *Staphylococcus aureus* genome. Derived sequences are submitted to the MLST database for identification. Known variants of each gene are thereby ascribed allelic numbers, which are then concatenated to form unique allelic profiles called 'sequence types' (ST). Sequence types that share at least 5 of the 7 alleles are called clonal complexes (CC). SCC*mec* typing classifies distinct allotypes of the gene and 11 have been described so far. Current MRSA nomenclature is to combine the MLST type (or CC) with the SCC*mec* type. EMRSA-15 is ST22-IV, i.e. Multi-Locus Sequence Type 22 containing SCC*mec* IV and EMRSA-16 is ST36-II. Different MRSA types are prevalent in different parts of the world, although some have spread internationally.

MRSA colonisation precedes infection. Presently in the UK, initial colonisation of the nose, throat, skin, gut and other sites almost always occurs in hospitals or other healthcare settings by cross-transmission from other patients who are infected or

asymptomatic. The organisms are usually transferred on contaminated staff hands, or less commonly, via the air or on fomites (Cookson *et al.,* 1989). Airborne transmission appears to be less common than with meticillin-sensitive strains. Nasal carriage by staff members is usually low at approximately 1–8% (Cookson *et al.,* 1989). The risk of colonisation and infection with MRSA increases with the length of hospitalisation, severity of underlying disease, number of operations or manipulations, and previous exposure to antibiotics, especially cephalosporins and aminoglycosides (Thompson RL *et al.*, 1982; Klimek JJ *et al.*, 1976). Colonisation may persist for months (Sanford MD *et al.,* 1994), including after hospital discharge to the community or other institutions where transmission can continue to occur. Such colonised patients may be re-admitted to hospital, which means that patients may be harbouring MRSA at the time of admission to hospital. This 'revolving door' epidemiology is one reason for the continuing epidemic of HA-MRSA (Robotham JV *et al.*, 2007). Thus, although some patients may appear to have community-acquired MRSA (CA-MRSA), they usually have a history of previous healthcare contact (Folden DV *et al.,* 2005; Tacconelli E *et al.*, 2004).

MRSA infections have higher mortality rates than those due to MSSA and are associated with increased morbidity, prolonged hospital stay and increased healthcare costs (Cosgrove SE *et al.*, 2003; Engemann JJ *et al.*, 2003; Stevens DL *et al.*, 2002; Li Z *et al.*, 2001; Chief Medical Officer Winning Ways, 2003). In England between 1993 and 2006 the number of death certificates mentioning *S. aureus* increased from <100 to >2000 per year. Of these, the number specifying MRSA increased from about 50 to about 1650 per year (Office for National Statistics, 2006). The number giving MRSA as an underlying cause of death rose from about 5 to about 500 (Office for National Statistics, 2008); however, the total number of deaths mentioning MRSA fell slightly in 2007 to about 1600. Death certification is inexact, but these figures show that MRSA has been a significant and increasing cause of death in the UK. The reduction of healthcare-associated MRSA infections is a government and Department of Health priority.

The Department of Health (England) has produced a number of documents giving guidance on healthcare associated infections, including MRSA, and showing the central importance of this issue in the NHS strategy. These include: *Getting Ahead of the Curve* (2002), *Winning Ways: working together to reduce health care associated infection in England (*2003), *Towards Cleaner Hospitals and Lower Rates of Infection: a summary of action* (2004), *Saving Lives: a delivery programme to reduce health care associated infection including MRSA* (2005), *Essential Steps to Safe Clean Care: reducing health care associated infection* (2006), *Infection control guidance for care homes* (2006), *The Code of Practice for the prevention and control of HCAI* (2006), *Saving Lives: reducing infection, delivering clean and safe care* (2007), *Clean, safe care: reducing infections and saving lives* (2008) (Department of Health, 2002; Department of Health, 2003; Department of Health, 2004; Department of Health, 2005; Department of Health, 2006; Department of Health, 2006; Department of Health, 2006; Department of Health**,** 2007; Department of Health, 2008). These were followed in 2006 by the introduction of The Health Act, which included a Code of Practice for the prevention, and control of Health Care Associated Infection. This Act made the control of HCAIs, including MRSA, a legal responsibility of Chief Executives of healthcare facilities in England. The 2006 Act has since been superseded by the Health and Social Care Act 2008, but the section on HCAI had been little changed. The Health and Social Care Act was further revised in 2009.

The Code of Practice (2006) refers specifically to MRSA and national guidance on its control (Coia JE *et al.*, 2006) and states that hospitals and healthcare facilities must ensure that they have policies for: pre-admission/admission screening for early identification of carriers, decontamination procedures for colonised patients; isolation of infected or colonised patients; transfer of infected or colonised patients within NHS bodies or to other health care facilities and antibiotic prophylaxis for surgery. In 2007 the Department of Health produced a specific guidance document on MRSA screening: '*Screening for meticillin-resistant Staphylococcus aureus (MRSA) colonisation. A strategy for NHS trusts: a summary of best practice* (Department of Health, 2007).

1.1.2 MRSA Screening

Many interventions for MRSA control have been proposed, although the evidence base for many of them is poor (Coia JE *et al.*, 2006). Widely accepted practice includes staff hand decontamination to prevent transmission from patient to patient; cleaning to reduce environmental contamination; good hygienic practice, especially around wound dressing and intravascular catheter insertion and care; and isolation of both MRSA infected patients and asymptomatic carriers.

It is generally accepted that carriers should be identified by screening, and isolated and decolonised to prevent spread. It is accepted that decolonisation i.e. complete eradication of MRSA may not be achieved but that the therapy may reduce the bacterial load of MRSA. However, the effect of the therapy upon an individual is unpredictable and is influenced by a number of factors such as the presence of a colonised wound, when failure is more likely. Therefore, despite the different meanings, the terms 'decolonisation therapy', 'eradication therapy' and 'suppression therapy' are often used interchangeably in the infection control literature (for the remainder of this thesis the term 'decolonisation therapy' will be used). Carrier surgical patients can be decolonised and offered anti-MRSA surgical antibiotic prophylaxis to reduce MRSA surgical site infections (SSIs). However, screening is costly and its exact role has not been defined. Despite clear World Health Organisation (WHO) criteria for screening programmes (Wilson JM & Jungner YG, 1968) there are many features of MRSA screening which are undecided. There is a continuing debate amongst Medical Microbiologists about which patient groups should be screened and at which anatomical sites. The optimal method of MRSA screening is also undecided.

Many governments have increased the pressure on healthcare providers to reduce MRSA rates. The Departments of Health for England and Wales, and Scotland, as well as federal authorities in the United States, have legislated for mandatory screening for MRSA **(**The General Assembly of Pennsylvania House Bill No.700, Public Act 095-0312 Illinois Senate Bill, Senate No. 2580, State of New Jersey). In England there is a mandatory requirement for Hospital Trusts to report their MRSA

bacteraemia rates and achieve targets for year on year reductions, with penalties for those who fail (HPA CDSC DoH MRSA Surveillance system results accessed at http://www.hpa.org.uk/web/HPAweb&HPAwebStandard/HPAweb_C/1233906819629). These rates are published on the internet and there is a clear political and public perception that higher rates are associated with worse infection control and clinical practice. After much active campaigning in the United States, including by the Committee to Reduce Infection Deaths (RID), the government health insurance agency Medicare no longer reimburses hospitals for the higher costs associated with treating patients for certain hospital-acquired MRSA infections, thus applying pressure on healthcare providers from another angle **(**Centers for Medicare and Medicaid Services accessed at http://www.cms.gov/apps/media/press/release.asp?Counter=3041&intNumPerPage=10&checkDate=&checkKey=&srchType=1&numDays=3500&srchOpt=0&srchData=&srchOpt=0&srchData=&keywordType=All&chkNewsType=1%2C+2%2C+3%2C+4%2C+5&intPage=&showAll=&pYear=&year=&desc=&cboOrder=date).

1.1.2a Which patients to screen?

Screening of relevant admissions has recently become policy in England (Department of Health, 2007). Risk-based assessment remains in Wales (Department for Health, Social Services and Children, 2008) and Northern Ireland (Department of Health, Social Services and Public Safety, 2008), and in Scotland the relative benefits of universal screening versus a risk-based approach are being assessed (NHS Scotland, 2011). Before the introduction of a systematic screening programme, patients were identified as MRSA positive from clinical specimens such as wound swabs. It was eventually realised that clinical specimens only identify 12-18% of carriers, and this was referred to as the 'tip of the iceberg' (Salgado CD and Farr BM., 2006; Harbarth S *et al.*, 2006; Robiscek A *et al.*, 2008). Identification of MRSA colonised patients using clinical specimens alone clearly leaves the larger population of asymptomatic carriers unidentified and therefore without control measures. These patients remain at risk of developing MRSA infection themselves and are the reservoir for the on-going transmission of MRSA to others.

When screening was first introduced it was widely recommended that patients at risk of MRSA carriage be screened at the time of, or before, admission to hospital (Ritchie K *et al.*, 2006; Rubinovitch B & Pittet D, 2001; Coia JE *et al.*, 2006; Duckworth G *et al.*, 1998; Mulligan ME *et al.*, 1993; Muto CA *et al.*, 2003). Such an approach uses epidemiological factors such as age, frequency and timing of previous hospital attendances, and the patient's place of residence (e.g. a long term care facility or a privately owned home) to stratify patients as either high or low risk for MRSA carriage (Coia JE *et al.*, 2006). Those classed as being at high risk are screened for MRSA carriage on admission to hospital and enhanced MRSA control measures are instituted for those who are identified as MRSA positive. Colonised and infected patients should be isolated with contact precautions, staff should wear disposable gowns and gloves for each patient contact and patients should be prescribed MRSA decolonisation treatment (though the latter is not universally recommended). Staff should decontaminate hands between each patient contact, and the environment should be appropriately cleaned (Coia JE *et al.*, 2006). Though the effectiveness of this screening strategy has not been confirmed, it has been widely adopted.

Screening only high risk patients rather than screening all patients was felt to be a pragmatic and cost-efficient approach to MRSA control at the time of the emergence of MRSA. Despite these efforts, MRSA is now endemic in UK hospitals. Risk-based approaches miss cases of MRSA carriage; in one study which limited MRSA screening to patients with at least one risk factor for MRSA, 65.2% of patients would be targeted and 87.5% of MRSA carriers identified (Lucet JC *et al.*, 2003). If screening was limited to patients with ≥ 3 risk-factors, 3.6% of patients would be screened and 18.6% of MRSA cases detected. This evidence, which informed conclusions drawn by a Scottish Health Technology Assessment of the positive benefits of universal screening (Ritchie K *et al.*, 2007) coupled with the success of a 'Search and Destroy' policy in the Netherlands and the experience of NHS trusts that had already implemented more extensive screening led to the introduction of universal screening of all patients regardless of their risk profile (Duerden B, 2008a; Duerden B, 2008b). Furthermore, with selective screening staff may fail to screen all patients at risk for reasons of forgetfulness, lack of time or misunderstanding, a situation avoided by universal screening. However the decision to change to universal

screening is controversial since the continuing endemic MRSA situation in UK hospitals is probably due more to poor compliance with infection control practices than failure to identify all colonised patients and because universal screening has considerable staffing and financial implications. Furthermore, the extension of universal screening to cover low risk patients inevitably lowers the screening pick up rate and cost effectiveness.

1.1.2b Which anatomical sites to screen

The choice of anatomical sites to screen for the optimal detection of MRSA carrier patients is undecided. One outbreak study reported that screening at the anterior nares picked up 78.5% of the positive patients who were identified by screening at multiple anatomical sites. The combination of nose and throat swabs identified 85.6% of such patients, the nose and perineum 93.4% and screening at the nose, throat and perineum identified 98.3% of them (Coello R *et al.*, 1994). However, screening of the throat and perineum poses logistical problems and the groin is often used as a surrogate for the latter. It is thought that perineal carriers of *S. aureus* may be heavier dispersers than nasal carriers, which results in airborne spread (Bøe J *et al*., 1964; Williams REO 1963). However, these patients are not subject to more rigorous or different infection control measures than patients who are not perineal carriers. The greatest multiplication of *S. aureus* is thought to occur in the nose, as the application of penicillin here reduces the quantity of *S. aureus* on the skin (Williams REO, 1963). However, the throat is an important carriage site as it may be the site for recolonisation of the nose following antimicrobial therapy (Casewell MW & Hill RLR, 1986). It has also been reported that MRSA carriers, colonised patients and those with infection differ in their carrier sites (Coello R *et al.*, 1994). Carriers were more likely to be positive at the nose than colonised and infected patients.

There are also differences in the yield of MRSA from different sites and in different types of patients. For example, a recent study on ICU patients demonstrated that the throat and rectum are significant reservoirs of MRSA, and carriers would be missed by screening only at the nose, axilla and groin (Batra R *et al.*, 2008). This is notable as perineal carriage was previously thought to be uncommon (Bøe J *et al*., 1964).

Despite the improved yield of MRSA detection by screening at more than one anatomical site, many healthcare providers continue to screen at the anterior nares only. The reasons for this are an assumed better compliance with screening if there are fewer sites to swab, and cost-efficiency, since the number of swabs and agar plates, and the amount of manpower required to process multiple swabs is much greater than for one. Some centres uses pooled multiple swabs to reduce the laboratory processing costs (Grmek-Kosnik I *et al.*, 2005).

1.1.2c Hospital versus community acquired strains of MRSA

Until recently, MRSA infections presenting outside of hospitals were caused by MRSA strains acquired during previous hospital or healthcare contact (Tambyah PA *et al.*, 2003; Habib AG *et al.*, 2006). True CA-MRSA caused by strains distinct from HA-MRSA in patients without prior healthcare contact began to emerge in the 1990s (Zetola N *et al.*, 2005). These clones are genetically distinct from hospital strains and appear to have emerged by acquisition of the SCC*mec* cassette by community strains of MSSA (Zetola N *et al.*, 2005). While HA-MRSA strains tend to cause infection in hospitalised, compromised, elderly patients, often with a history of surgery or indwelling devices, CA-MRSA, like community strains of MSSA, affect younger, healthy people and can spread readily in community settings and hospitals. Unlike HA-MRSA, but like the MSSA strains they are derived from, CA-MRSA are often virulent, causing primary skin infections and invasive sepsis in healthy people. CA-MRSA are characteristically susceptible to most non-β-lactam antimicrobial agents, contain SCC*mec* types IV or V and frequently produce the Panton-Valentine Leukocidin toxin (PVL), a putative virulence factor (Vandenesch F *et al.*, 2003; Genestier AL *et al.*, 2005). Although the role of PVL is debated, PVL-positive CA-MRSA has been associated with severe skin sepsis and fatal necrotising pneumonia (Centers for Disease Control and Prevention, 1999; Gillet Y *et al.*, 2002). Nevertheless, not all CA-MRSA produce PVL, some strains have become multiply antibiotic resistant and CA-MRSA is increasingly the cause of hospital outbreaks (Otter JA & French GL, 2006), so that the epidemiological distinction between HA- and CA-MRSA is becoming blurred.

The extent of CA-MRSA colonisation in the general population has not been fully quantified in the UK but it is much less than in the US and is regarded as uncommon **(**Society for General Microbiology, 2008). By testing all of the MRSA isolates routinely submitted to the *Staphylococcus aureus* reference Laboratory (SRL), the Health Protection agency had identified only 100 cases over the three years to November 2009 (http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/StaphylococcusAureus/GeneralInformation/staphFrequentlyAskedQuestions/#q20). This represents 0.005% of the MRSA isolates received by the SRL each year (Health Protection Agency, 2005). However the increasing incidence of CA-MRSA has been reported at the London hospital where the present study is set (Otter JA & French GL 2008a; Otter JA & French GL, 2008b). European CA-MRSA prevalence rates are low but increasing (except in Greece where it approaches USA levels) (Vandenesch F *et al*., 2003; Wannet WJB *et al*., 2005; Otter JA & French GL, 2008a; Otter JA & French GL, 2008b). However, in the United States CA-MRSA is widespread: such strains account for a large proportion of *S. aureus* infections presenting to US Emergency Departments (Moran GJ *et al*., 2006) and are now the commonest cause of both hospital and community *S. aureus* infections in certain US cities. A single successful clone, the PVL-positive USA300, dominates in the USA but CA-MRSA strains from most other parts of the world are characterised by clonal diversity with only about half expressing PVL (Otter JA and French GL, 2010). The problem of PVL-producing strains of both MRSA and MSSA is recognised as an important emerging national clinical issue (Nathwani D *et al*., 2008; Health Protection Agency, 2008). Although CA-MRSA has not been the focus of hospital MRSA control programmes, it is imperative that carriers of such strains can be identified by hospital MRSA screening strategies.

1.1.2d Nucleic acid amplification tests for MRSA screening

There is no consensus on the best method of MRSA detection to use for screening. Culture techniques are the staple of MRSA detection in hospital diagnostic laboratories. These tests take on average between 1 and 3 days of laboratory time to produce a result depending upon the method used. Even slower methods are also used.

It is assumed that faster detection of MRSA carriers on admission will lead to more rapid implementation of appropriate control procedures. This should result in lower hospital transmission rates and a few theoretical papers have made the case for this (Bootsma MCJ *et al.,* 2006; Cooper BS *et al*., 2004; Raboud J *et al.,* 2005; Kluytmans J, 2007).

Consequently a range of rapid polymerase chain reaction (PCR) screening tests for MRSA detection have been developed commercially and in-house which will produce a result in approximately two hours of laboratory time. They are based on the simultaneous amplification and detection of *mecA* and genes characteristic of *S. aureus*. However, meticillin-resistant coagulase-negative Staphylococci (MRCNS) also possess *mecA* and dual colonisation with MRCNS and MSSA is not infrequent: for example, this occurred in 3.4% of cardiothoracic patients studied in Germany (Becker K *et al.,* 2006). PCR systems that use unlinked primers targeting a *S. aureus* species-specific gene and *mecA*, such as the LightCycler *Staphylococcus* and MRSA detection kit (LC assay; Roche Diagnostics, Mannheim, Germany) and the Hyplex StaphyloResist PCR (BAG, Lich, Germany), may give false-positive results with such mixtures (Malhotra-Kumar S et *al.,* 2008).

To resolve this problem the BD GeneOhm™ system (previously known as IDI-MRSA™) (GeneOhm, San Diego, CA; BD Diagnostics) amplifies MRSA-specific sequences by targeting a single locus which includes the right extremity of SCC*mec* downstream of *mecA*, and part of the adjacent *S. aureus*-specific *orfX* gene. Five primers target SCC*mec* sequences corresponding to types I, II, III, IVa, IVb, and IVc,

and one primer and three molecular beacons are specific for *orfX* (Huletsky A *et al*., 2004). The test is performed in real time with fluorescence detection. However it is designed and licensed by the Food and Drug Administration (FDA) as a screening test for use only on nasal swabs. A similar assay is the GeneXpert MRSA (Cepheid, Sunnyvale, CA). The GeneXpert is a random access point of care test, which also targets the junction of the SCC*mec* and *orfX*. The screening test is licensed for use on nasal swabs and provides real-time results. It is designed for use by laboratory and non-laboratory (i.e. clinical) staff on the ward as a near patient test. It therefore does not need the high level of technical expertise that is required for the IDI-MRSA™ test. The turn-around time of the test from processing the specimen to result is reported by the manufacturer as 70 minutes.

For reasons of cost efficiency, batching of tests for processing by PCR is commonplace. However, a Scottish systematic review of MRSA screening (Ritchie K *et al*, 2007) concluded, "In routine clinical practice, where samples require to be batched before testing, PCR does not have a turnaround time advantage over chromogenic agar testing." Many screening swabs can give a same day result when processed by present PCR systems, but in routine practice a significant proportion will have a result the day after sampling. Furthermore, good clinical microbiological and infection control practice requires that positives cases will then need culture (or re-swabbing and re-culture) to confirm antimicrobial susceptibilities and other tests for treatment and epidemiological reasons as well as MRSA confirmation.

1.1.2e Cost of MRSA screening

The costs of PCR tests for MRSA screening are significantly higher than for conventional culture. It is difficult to define the exact costs because reagent and equipment costs differ with rental packages, quantity discounts and currency rates. However, a Scottish systematic review (Ritchie K *et al.*, 2007) calculated that the cost for a screening test done with chromogenic agar, latex agglutination and a confirmatory disc test was £4.35 for a negative test and £7.45 if positive, and the cost for the IDI-MRSA™ real time PCR test was £19.40 for both positive and negative specimens. The IDI-MRSA™ test in the present study was approximately £10.50 for

consumables alone. It is possible that the IDI-MRSA™ may cost more when used for diagnostic (and not research) purposes. The comparator conventional culture method used in this study, an MRSA selective broth (made in-house) coupled with a Chromagar (Oxoid, Basingstoke, UK) costs approximately £2.00 for consumables. For this culture screen, specimens from the nose, axilla and groin of each patient are pooled together in one broth to improve cost-efficiency. Because of the more rapid results, the increased costs of IDI-MRSA™ might be offset by savings resulting from reduced cross-infection, fewer complications and better bed utilisation. A recent study in the Netherlands (Wassenberg MWM *et al*., 2010) defined the costs in their institution as follows:

	BD GeneOhm MRSA PCR	Xpert MRSA assay	MRSA-ID, bioMérieux (culture)
Cost per test	30.49€	42.84€	1.40€
Additional costs	0.75€	0.75€	0.68€
Platform costs	16.01€	23.93€	Not applicable
Personnel costs	8.97€	2.10€	Included in additional costs
Total cost per unit	56.22€	69.62€	2.08€

The place of PCR screening in the hospital is not straightforward and these tests need clinical and financial justification by testing in real life, operational settings. This thesis presents two papers investigating PCR detection for MRSA. One is a validation of the IDI-MRSA™ for use on pooled nose, axilla and groin swabs, and specimens from other anatomical sites. The second, larger paper is a randomised controlled trial to investigate the clinical efficacy of IDI-MRSA™ for MRSA screening.

Chapter 2: The pooling study materials and methods

2.1 Summary

A commercial rapid PCR MRSA screening method (IDI-MRSA™) has been validated for the use with nasal swabs transported in liquid Stuart's medium. The IDI-MRSA™ was investigated for use for MRSA screening in pooled nose, axilla and groin swabs and in single swabs from skin puncture sites, wounds, throat, rectum and groin, using swabs transported in Amies' medium without charcoal. Amies modified Stuart's medium by 1) replacing the glycerophosphate with an inorganic phosphate buffer, 2) adding calcium salts, magnesium salts and charcoal, 3) increasing the agar concentration and 4) removing the methylene blue (Amies CR. 1967). The purpose was to improve the survival of pathogens and prevent the overgrowth of commensals. The IDI-MRSA™ test was applied to swabs that had been used for routine MRSA broth culture and which were selected to be about 50% MRSA positive. It was compared with conventional MRSA culture screening.

2.2 Objectives

This study aimed to validate the IDI-MRSA™ test on non-nasal and pooled specimens prior to use of the test in this way in a randomised controlled clinical trial. The IDI-MRSA™ is licensed for use on swabs taken from the anterior nares, which have been transported in liquid Stuart's medium. Thus we were validating this test for use in an off licence application.

2.3 Setting

The study was carried out at Guys' and St. Thomas' NHS Foundation Hospital Trust (GSTT), a 1200 bedded teaching and tertiary referral acute Hospital Trust in central London. The Trust has a wide range of specialities at particular risk of MRSA colonisation and infection, including intensive care and high dependency units, and renal, haematology, oncology, cardiac, vascular, orthopaedic and dermatology units. The Trust admits many patients at risk of MRSA carriage, including re-admissions

and those from other hospitals, elderly care homes and from abroad. Because of this, GSTT has an active MRSA surveillance programme, including MRSA screening.

2.4 Specimens

Patients were routinely screened for MRSA by swabbing the nose, axilla and groin. Three separate swabs were used, one swab for both anterior nares, one for both axillae and one for both groins. The swabs were transported in Amies' medium (without charcoal) (Barloworld Scientific, Stone, UK) and pooled for culture; they were designated 'pooled' screening swabs. Some patients had additional, separate, single swabs taken from throat, rectum, perineum, wounds and/or skin puncture sites such as stomas, suprapubic and intravascular catheter sites; these were designated 'other site' screening swabs and were cultured and analysed separately. Each day we identified culture positive MRSA screening swabs from the laboratory computer system, retrieved them from the 4°C refrigerator where they had been stored in the laboratory and processed them using the IDI-MRSA™ test. Culture negative MRSA screen specimens were chosen at random to make up about 50% of the total number of specimens tested. This was a validation study which started with the processing of MRSA culture positive swabs. Therefore the initial selection of swabs was biased towards those with an MRSA positive result. For this reason, a power calculation was not performed prior to the study.

2.5 Routine culture method

An in-house MRSA selective broth (MRSA broth) was used for routine culture of screening specimens and was based on those described by others (Gurran C *et al*., 2002; Kelly S *et al*., 2004). The MRSA broth contained 25g nutrient broth (Oxoid, Basingstoke, U.K.), 5g mannitol (BDH Ltd, Poole, U.K.), 5g trehalose (Merck Sharp & Dohme, Ltd. Hoddesdon, U.K.), 30g sodium chloride (BDH Ltd, Poole, U.K.), 700µl 2% phenol red indicator (BDH Ltd, Poole, U.K.), 4ml ciprofloxacin (8mg/L) (Bayer, Newberry, U.K.), 10ml aztreonam (20mg/L) (Bristol-Myers Squibb, Uxbridge, U.K.) and 1ml colomycin (1 000 000 U/L) (Forest Laboratories Inc,

Bexley, U.K.) in 1 litre of deionised water. Pooled nose, axilla and groin swabs from each patient were swirled in 3ml of MRSA broth. Swabs from other sites were processed in individual broths (also 3ml). After 18-24 hours of aerobic incubation at 35-37°C, a red colour broth indicated that MRSA was not present and the result was reported as 'MRSA not detected'. An orange ('borderline') or yellow ('positive') colour change suggested the presence of MRSA and a direct tube coagulase test was performed on the broth. Broths with a positive tube coagulase reaction were sub-cultured on to mannitol salt agar (Oxoid, Basingstoke, UK); those with a negative reaction were sub-cultured to Columbia blood agar (Oxoid, Basingstoke, UK). Suspected *Staphylococcus aureus* isolates were confirmed by Gram stain and tested for catalase production, detection of the fibrinogen affinity antigen (clumping factor), protein A and the capsular polysaccharides of *S. aureus* using Pastorex® (Bio-Rad, Hemel Hempstead, U.K.), and mannitol fermentation. Susceptibilities to meticillin and other antibiotics were determined according to BSAC disc diffusion methods (Andrews JM *et al*., 2005).

2.6 IDI-MRSA™ analysis

The IDI-MRSA™ method was a variation of that described by Warren and colleagues (Warren DK *et al*., 2004) with reagents and some equipment supplied by GeneOhm Sciences Ltd (Sheffield, UK).

Pooled nose, axilla and groin screening swabs. Each swab was put into a tube containing 0.3ml of sample buffer: one tube contained one swab. The swab stem was broken off, leaving the tip inside the tube, which was then closed tightly and vortexed at high speed (2700 rpm) for 1 minute. The buffer suspensions from the three samples (0.3ml x 3) were then combined into a single lysis tube (which contained glass beads in order to lyse the cells) which was heated at 95°C for 2 minutes to liquefy any Amies' medium gel and facilitate its removal later. The tube was then centrifuged for 5 minutes at 14000 rpm at room temperature. The supernatant was discarded and 50µl of sample buffer added. The tube was vortexed for 5 minutes, followed by heating to 95°C for 2 minutes and then a short (approximately 5 seconds) pulse centrifuge at room temperature to settle the fluid. The lysis tubes were kept on ice or a cooling

block (at 4°C) until ready for the amplification step. The PCR was performed according to the manufacturer's instructions by using a Smart Cycler II device (Cepheid, Sunnyvale CA, U.S.A.) which has the capacity to process 14 specimens (plus 2 controls) simultaneously. Specimens inhibited on initial PCR were re-amplified.

2.7 Culture negative/IDI-MRSA™ positive results

We reviewed previous microbiology results for patients with culture negative/IDI-MRSA™ positive results in order to determine whether or not they had previously been colonised or infected with MRSA. We did not have ethical approval to re-swab patients and re-test them by either method, nor did we review subsequent results for these patients to see whether or not they developed MRSA positive culture tests after the culture negative/IDI-MRSA™ positive result.

Chapter 3: The pooling study results

3.1 Pooled nose, axilla and groin screening swabs

A total of 203 sets of pooled nose, axilla and groin swab sets were tested. Due to the requirement to await a finalised culture result and the logistics of the workflow in the department, MRSA positive swabs were stored at 4°C for 3-5 (mean 3.9) days and the MRSA negative swabs for 7-11 (mean 9.2) days prior to IDI-MRSA™ testing. Three sets (1.5%) were initially inhibited and required re-testing. Two culture positive specimens (0.9%) were unresolved (i.e. a negative or positive result could not be obtained due to repeated inhibition of the reaction despite an overnight freeze-thaw cycle as recommended by the manufacturer) and eliminated from the analysis, leaving 201 sets, of which 99 (49.3%) were MRSA culture positive (Table 1). Eighty-four (84.8%) of the 99 culture positive sets were IDI-MRSA™ positive and 97 (95.1%) of the 102 culture negative sets were IDI-MRSA™ negative. The sensitivity of the IDI-MRSA™ in comparison with culture was 84.8%, the specificity was 95.1%, the negative predictive value (NPV) was 86.6% and the positive predictive value (PPV) was 94.4%(Table 1). The five specimens that were MRSA culture negative/IDI-MRSA™ positive came from five patients, all of whom had previously been identified as MRSA positive within the previous month. After including the results of these patients as culture positive and IDI-MRSA™ positive (referred to as 'resolution' of the discrepant result), the sensitivity was 85.6%, the specificity was 100%, the NPV was 86.6% and the PPV was 100%,

3.2 'Other Site' single screening swabs

There were 32 swabs in this group. MRSA positive swabs had been stored at 4°C for 3-11 (mean 7.2) days and all of the MRSA negative swabs for 3 days prior to IDI-MRSA™ testing. No specimen showed inhibition. Of 17 swabs that were MRSA culture positive, 16 (94.1%) were IDI-MRSA™ positive (Table 2). Of the 15 swabs that were culture negative, 12 (80.0%) were IDI-MRSA™ negative. The sensitivity of the IDI-MRSA™ in comparison to culture was 94.1%, the specificity was 80%, the NPV was 92.3% and the PPV was 84.2% (Table 2). Two of the three patients with culture negative/IDI-MRSA™ positive results were known to have been MRSA

positive within the month prior to the discrepant sample. The other patient did not have any other cultures for MRSA. After including two of these results as culture positive and IDI-MRSA™ positive the sensitivity was 94.7%, the specificity was 92.3%, the NPV was 92.3% and the PPV was 94.7%,

3.3 Turn-around Times

The turnaround time for one batch of 14 pooled swabs was approximately 2 hours.

Table 1 Pooled IDI-MRSA™ screening

		MRSA Culture		
		Positive	Negative	Total
IDI-MRSA™	Positive	84 (84.8%)	5	89
result	Negative	15	97 (95.1%)	112
	Total	99 (49.3%)	102 (50.7%)	201

Table 2 Single swab screens from other sites

		MRSA Culture		
		Positive	Negative	Total
IDI-MRSA™	Positive	16 (94.1%)	3	19
result	Negative	1	12 (80.0%)	13
	Total	17 (53.1%)	15 (46.9%)	32

Chapter 4: The pooling study discussion

The pooling study discussion

Patients are routinely screened for MRSA carriage from swabs taken from the anterior nares. These swabs will identify approximately 80% of MRSA carriers (Coello R *et al*., 1994). The likelihood of identifying MRSA carriers increases with the increasing number of sites screened. Many but not all centres also process specimens taken from other anatomical sites such as the throat, perineum, groin, axilla and skin breaks. The combination of nose, throat and perineum seems to have the highest yield at 98.3% (Coello R *et al*., 1994). Many screen at the nose, axilla and groin because of the practical difficulties of swabbing the throat and perineum. However many others still screen patients for MRSA just at the anterior nares, usually for reasons of cost.

GSTT has a policy of detecting MRSA by culture from pooled specimens taken from the nose, axilla and groin. Specimens from other anatomical sites are processed individually. The method of MRSA detection is a selective MRSA enrichment broth made in-house. This method is used because it is accurate and pooling makes the process more cost-efficient than other methods. Swabs are transported to the laboratory in Amies' medium without charcoal. The IDI-MRSA™ test is licensed only for use on swabs taken from the anterior nares transported in liquid Stuart's medium (IDI-MRSA™ test product insert); it is not validated for use on specimens from other sites, or for use on pooled specimens, or for use on specimens transported in Amies' medium. For our proposed trial of this PCR screening system, and for reasons of cost-efficiency, specimens from the nose, axilla and groin would ideally be pooled before processing by IDI-MRSA™ and specimens would still be transported in Amies' medium. We therefore needed to validate the IDI-MRSA™ system for use with these pooled swabs before starting our clinical trial.

Compared with culture results for the pooled nose, axilla and groin swab sets, in our study the IDI-MRSA™ system had a sensitivity of 85% and a specificity of 95%. The sensitivity of IDI-MRSA™ for the detection of MRSA from nasal swabs alone has been reported by others as between 90.0% and 91.7% (Bishop *et al*., 2006; De San N *et al*., 2007; Reyes R *et al*., 2006; Warren DK *et al*., 2004; Zhang XS *et al*., 2007) and the specificity as between 91.7% and 97.1% (Bishop E *et al*., 2006; De San N *et al*.,

2007; Reyes R *et al*., 2006; Warren DK *et al*., 2004; Zhang XS *et al*., 2007). The sensitivity and specificity of IDI-MRSA™ for pooled nose, axilla and groin swabs (transported in Amies' media) when compared to culture, are therefore similar to the same comparison for nasal swabs alone. By testing swabs from the nose, axilla, groin, skin breaks and other clinically relevant specimens, we should be detecting more MRSA carriers than those testing at only the nose (Hombach M *et al*., 2010). Therefore in terms of MRSA control, the reduction in sensitivity of 5% from 90% to 85% is not as it would seem. Thus we conclude that the use of IDI-MRSA™ with pooled nose, axilla and groin swabs is acceptable for routine use.

There have been other recent reports on the use of IDI-MRSA™ with pooled MRSA screening swabs. Drews and colleagues analysed 164 pooled nasal, axilla, groin and perineum swab sets and found a sensitivity of 96% and a specificity of 94% (our calculation from their data) (Drews SJ *et al*., 2006). In the same study, 21 pooled axilla and groin swabs had a sensitivity and specificity of 100%. Nasal and rectal swabs pooled in broth and incubated overnight prior to IDI-MRSA™ testing also performed well, though after introduction as a diagnostic test, the positive predictive value fell from 90% to 65% and this group now confirms all such IDI-MRSA™ positive samples with culture (Desjardin M *et al*., 2006). Bishop and colleagues reported a sensitivity of 88% and specificity of 91.6% for IDI-MRSA™ when used on 192 pooled nose and groin swab sets, and MRSA detection by IDI-MRSA™ was similar for single and pooled swabs from the same sites (Bishop E *et al*., 2006).

All of the quoted studies processed each specimen by both culture and IDI-MRSA™. The majority cultured on to solid media before IDI-MRSA™ testing; Desjardins and colleagues cultured specimens in a tube of selective broth, which was incubated overnight before a 50µl aliquot was tested by IDI-MRSA™ (Desjardins M *et al*., 2006). Three studies (Bishop E *et al*., 2006; De San N *et al.,* 2007; Warren DK *et al*., 2004) inoculated an enrichment broth after IDI-MRSA™ testing. One study re-cultured the swabs and the transport media of discrepant specimens, using enrichment broth for the former (Drews SJ *et al*., 2006). In the present work, we analysed swabs that had previously been used for broth culture, which may have reduced the number of bacteria present for subsequent IDI-MRSA™ analysis. Furthermore, in order to

select an appropriate number of MRSA culture positive and negative samples for analysis, the swabs were stored whilst awaiting culture results prior to IDI-MRSA™ testing. These factors may have reduced the sensitivity of IDI-MRSA™. However it is plausible that bacteria remained on the swabs prior to testing by IDI-MRSA™ and that the negative IDI-MRSA™ tests were not due to a low number of bacteria in the clinical specimen. It is also possible that the bacteria multiplied on the swab pending processing by PCR. The negative PCR results may be due to the use of Amies' transport medium. One group performed a validation study of the IDI-MRSA™ test for use on multiple specimen types including groin and wound swabs. The comparator was enrichment culture of the swab tip which had already been processed by the IDI-MRSA™ test. Enrichment broth was added to the sample buffer tube which contained the tip and this was incubated aerobically at 35°C for 24 hours. Approximately 100µL was sub-cultured on to chromogenic agar. The sensitivity of the IDI-MRSA™ test was 81.7%, which increased to 84.3% after resolution of discrepant results by searching for a history of MRSA decolonisation, reviewing the molecular size of the PCR product, or finding evidence of other MRSA positive results for the same patient at the same time (Lucke K *et al*., 2010). These sensitivity values are comparable to the sensitivity in the present study. However, when the same group used the IDI-MRSA™ test on the same specimen types transported in liquid Stuart's medium, the sensitivity was 100%. As in the present study, it is possible that the use of an agar-based medium compromises the elution of staphylococci (Hombach M *et al*., 2010). However, it is not clear why the IDI-MRSA™ and not the culture test would be affected if this was the case.

In the present study we also analysed 32 separate swab screens from 'other sites'. For these specimens the sensitivity of the IDI-MRSA™ in comparison to culture was 94.1% and the specificity 80%. In the study by Drews and colleagues of 122 non-pooled screening swabs from perineum, rectum and wounds, the IDI-MRSA™ had a sensitivity of 95% and a specificity of 90% compared with culture (the sensitivity and specificity rose to 97% after the inclusion of other MRSA results) (our calculation from their data) (Drews SJ *et al*., 2006). De San and colleagues tested 997 non-pooled swabs taken from the nose, throat, perineum and wounds and found that, compared with culture, IDI-MRSA™ had a sensitivity of 81%; the sensitivity for nasal swabs alone was 90.6% (De San N *et al*., 2007). Another group found a sensitivity of 94.8%

and a specificity of 96.0% when 987 rectal swabs were tested, and a sensitivity of 100% and a specificity of 93.6% when testing 152 'other site' swabs (Zhang XS *et al*., 2007). Reyes and colleagues compared IDI-MRSA™ with chromogenic MRSA selective media for the detection of MRSA from rectal swabs: IDI-MRSA™ had a sensitivity of 82% and a specificity of 99%. The reason for the variation in sensitivity is not clear, although the inclusion of all of the MRSA culture results for each patient would improve the sensitivity for our data as it does for others (Drews SJ *et al*., 2006). With these caveats, and although the number of specimens that we tested is relatively small, we concluded that IDI-MRSA™ is able to reliably detect MRSA from individual swabs of sites other than the nose.

Only three screening samples (all pooled sets) had inhibited PCR reactions and required re-amplification by IDI-MRSA™. This represented only 1.3% of all tests in this study, which is three and a half times lower than the manufacturer's published rate of 4.5% (IDI-MRSA™ Test Product Insert), three times lower than that reported for pooled nose and groin swabs (4%) (Bishop E *et al*. 2006), and four times lower than that reported for both pooled nasal, axilla, groin and perineum swabs, and for pooled axilla and groin swabs (5%) (Drews SJ *et al*., 2006). We had anticipated higher rates of inhibition due to the processing of swabs taken from sites with a greater number of inhibitors; the axillary swabs may have deodorant on them, perineal swabs will have a greater numbers of organisms from the lower gastrointestinal tract and wound swabs may have other skin flora as well as topical therapies and dressings. Inhibition rates have been reported as approximately 12% each for perineal swabs and rectal swabs (Drews SJ *et al*., 2006) and 9% for wounds (Drews SJ *et al*., 2006). It has been suggested that the volumes of sample buffer used in pooling may dilute inhibitors in the specimen (Bishop E *et al*., 2006; Desjardins M *et al*., 2006). However, the quantities of buffer that we used were the same as in the manufacturer's validated assay and none of our 'other site' non-pooled swabs were inhibited. Substances that inhibit PCR reactions are often temperature sensitive and a common approach to resolving inhibited PCR tests is to freeze the DNA extract overnight, thaw the follow day and attempt the amplification step again. Inhibitors also degrade with time and it is possible that in the present study, one or both of refrigerating the specimens until they were processed by IDI-MRSA™ and storing them until

processing contributed to the low inhibition rate. It is also possible that our low initial inhibition rate compared with other studies may have resulted from the extra heating step we used to liquefy the transport gel but which may also have removed PCR inhibitors. Heat treatment has been shown to reduce the inhibition rate of PCR for detection of *Chlamydia trachomatis* nucleic acid from cervical swabs (Verkooyen RP *et al*., 1996) and the inhibitory effect of bile on PCR (Al-Soud WA *et al*., 2005). Uniformity of temperature within the reaction chambers is important for the performance of PCR tests and a slightly higher sensitivity of the IDI-MRSA™ assay has been found with the Rotor-Gene 6000 in comparison to the Smart Cycler perhaps because the latter uses warmed air to reduce between-tube temperature variability (Smith MH *et al*. 2010) However the study presented in this thesis used the Smart Cycler system. The difference in PCR inhibition rates between the 'other site' swabs and the pooled swabs may be due to the dilution of inhibitors as the latter were processed in 0.9ml of sample buffer and the former in 1ml. Any inhibitors would be at a higher concentration in the pooled swab specimens thus increasing the chance of PCR inhibition occurring.

Two inhibited specimens were unresolved after repeat testing, 0.9% of all tests. This is similar to the manufacturer's published rate of 1% (IDI-MRSA™ Test Product Insert), and to other reports for pooled swabs (Bishop E *et al*., 2006; Desjardins M *et al*., 2006).

We calculated predictive values though the selection of swabs was biased because we chose them on the basis of the culture result. The positive predictive value (PPV) for pooled nose, axilla and groin swabs was 94.4%, and it was 84.2% for single swab screens. Of the specimens that were routine culture negative/IDI-MRSA™ positive, 88% were from patients who had previous MRSA culture positive results. After resolution of culture negative/IDI-MRSA™ positive results by searching for a history of MRSA colonisation, the PPV improved to 100% for pooled nose, axilla and groin swabs, and to 94.7% for single swabs. Other studies have found that between 40 and 70% of culture negative/IDI-MRSA™ positive patients had an MRSA history (Bishop E *et al*., 2006; Drews SJ *et al*., 2006). It is possible that these patients have a low-level of MRSA colonisation which was not detected by standard culture (Desjardins M *et*

al., 2006). The IDI-MRSA™ has been reported to have lower limits of detection than culture (Hombach M *et al*., 2010).; The negative culture screening result in these discrepant cases may have been due to the effect of decontamination therapy on the amount of MRSA present at the swabbed sites. However others have found that culture negative/PCR positive specimens were not associated with decontamination therapy (Herdman MT *et al*., 2009). Although the process of swirling the swabs in the broth is to elute bacteria from the swabs and in to the medium, some bacteria will remain on the stored swabs and will multiply in the time interval between storage at 4°C and IDI-MRSA™ testing. If organisms are present in low numbers at the time of culture, but in higher numbers at the time of IDI-MRSA™ processing, the culture result may be falsely negative and the IDI-MRSA™ test truly positive. Finally, these patients may have had a true IDI-MRSA™ false positive test. Meticillin susceptible *Staphylococcus aureus* isolates derived from MRSA lineages may contain residual fragments of SCCmec after deletion of the mecA gene and these strains have caused false positive IDI-MRSA™ assays (Desjardins M *et al*., 2006; Huletsky A *et al*., 2004). Similarly, strains of coagulase negative staphylococci which cross react with single-locus PCR tests designed to detect MRSA, have high homology to the *orfX-SCCmec* region in MRSA (Malhotra-Kumar S et al,. 2010)

The negative predictive value (NPV) for the pooled nose, axilla and groin swabs was 86.6% and for single swab screens was 92.3%. Culture negative/IDI-MRSA™ positive results were not investigated further in this study. Swabs which are MRSA culture positive and IDI-MRSA™ negative may contain MRSA undetectable by IDI-MRSA™. This may be because the MRSA strains are *mecA*-negative and are resistant to meticillin by β-lactamase hyper production (Chambers, 1997). Alternatively there may be variability of the *SCCmec* element or *orfX* (Huletsky A *et al*., 2004).
Our turn-around time of 2 hours for 14 sets of pooled swabs was very similar to that found for the validated assay (IDI-MRSA™ Test Product Insert) and confirms the rapidity of this assay.

In conclusion, despite the limitations of this study that tested stored swabs previously used for routine cultures and selected swabs on the basis of the MRSA culture result, we confirmed that the IDI-MRSA™ system is suitable for use on pooled nose, axilla

and groin swabs, and on single screening swabs from other sites. These swabs were transported in Amies' medium without charcoal, but this did not compromise the results when an extra heating step was included to liquefy the gel. Since multiple site swabs increase the yield of MRSA positive screens and pooling reduces costs, we concluded that we should use IDI-MRSA™ with these types of screens in our proposed clinical trial.

Chapter 5: The rapid MRSA screening study materials and methods

5.1 Summary

A randomised crossover trial was conducted to compare a rapid PCR-based test (using the IDI-MRSA^{TM} test) with conventional culture methods for the control of hospital acquired MRSA rates in an acute, NHS Trust. The primary outcome was the MRSA acquisition rate, expressed as the percentage of the patients included in the analysis who acquired MRSA after hospital admission. We also calculated the MRSA acquisition rate per 1000 patient-days at risk and measured the MRSA transmission rate. Secondary outcomes were MRSA bacteraemia and MRSA wound infection rates, resource use (use of isolation facilities), and test specific characteristics.

5.2 Objectives

The study was conducted at Guy's and St. Thomas' NHS Foundation Trust (GSTT), whose characteristics were described previously. Despite improving MRSA rates the Trust has endemic MRSA. We chose study wards that had clinical settings and MRSA transmissions typical of for the NHS at that time.

It is widely assumed that a more rapid MRSA screening result will facilitate the more rapid implementation of targeted MRSA control procedures and thus reduce MRSA transmission and infection rates. Commercial PCR screening tests are much more rapid than conventional culture screens, but much more expensive. The higher costs of the PCR method can only be justified in the NHS if the use of the test does indeed significantly reduce MRSA in an NHS setting when compared with conventional screening.

The study objectives were therefore to compare the efficacy of a rapid MRSA screening test with conventional culture methods for the control of MRSA in an acute, NHS hospital with endemic MRSA. Specifically, we measured the outcomes of MRSA acquisition and transmission rates, MRSA bacteraemia and wound infection rates, utilisation of isolation facilities, and test characteristics.

The IDI-MRSA™ test was one of a few, new 'rapid' diagnostic tests for the detection of MRSA colonisation and was one of the first licensed for use by the FDA. This was therefore chosen as the rapid test for this trial. It was compared with our conventional culture screen in a cross-over study.

The intention was that the results of this study would be generalisable to other UK NHS Trusts, and assist hospitals and the UK Government in policy decisions on MRSA screening. The study was funded by the Department of Health.

5.3 Study design

This was a randomised crossover trial of rapid PCR-based testing compared to conventional culture for all admissions to 10 acute wards with different specialities in an acute NHS Trust over a period of 15 months.

Randomisation was performed at ward level. This design was chosen because individual randomisation would not allow measurement of the specified outcome measures.

5.4 The trial

1. A randomised, unblinded, crossover trial conducted at GSTT.
2. Ten study wards: six surgical (plastic, urology, gastrointestinal, cardiothoracic, vascular, and ear, nose and throat), two elderly care wards and two oncology wards located across both Trust sites. All wards had endemic MRSA and already performed MRSA admission screening. The surgical wards had pre-admission clinics with pre-admission MRSA screening.
3. All patients admitted to the study wards were eligible for inclusion in the study.

4. Patients were screened on admission and at discharge in order to measure ward MRSA admission colonisation rates and ward acquisition and transmission rates.
5. After a baseline data collection period of 3 months, 5 of the wards were randomly allocated to the control or intervention phase for 5 months, which was followed by a one-month wash out period. The wards then crossed over for a second five-month period. A one-month data collection phase followed.

Wards were randomised to the intervention or the control. The intervention was the provision of IDI-MRSA™ in addition to conventional culture on MRSA admission screening specimens. After the crossover, the wards swapped the MRSA screening method. During all other phases including the control phase, MRSA admission screening was by conventional culture only. MRSA discharge screening was by culture during all periods.

5.4.1 Screening

5.4.1a Ward screening

1. With verbal consent, ward nurses screened patients for MRSA at or within 48 hours of the time of study ward admission (a widely accepted convention for infection control studies) and at or within 48 hours of study ward discharge (again, an accepted convention). Patients were also screened on the surgical admissions lounge (SAL) where some patients are seen before direct transfer to theatre, from where they were admitted to their study ward. Specimens taken within 48 hours after discharge i.e. after ward transfer were considered valid for inclusion.
2. Screening swabs were taken from the anterior nares, axillae, groins and skin breaks, such as wounds, ulcers and vascular catheter insertion sites, as well as other clinically indicated specimens such as urine, pleural fluid and sputum.
3. During the intervention phase, a duplicate set of admission swabs/specimens

was taken. One set was processed by MRSA culture, the other by IDI-MRSA™. The method for IDI-MRSA™ testing on pooled swabs and single swabs had been validated at GSTT on one set of stored swabs which had already been processed for MRSA detection by culture (Study 1), not on duplicate swabs.

4. The ITU routinely screens at the nose, throat and perineum and these swabs were considered valid for those taken up to 48 hours after discharge.
5. Where possible, discharge screening was performed on all patients using culture screening as described above.
6. Patients present in more than one phase were not re-screened

5.4.1b Specimen transportation

1. The Microbiology laboratories are located on the St. Thomas' Hospital site. The routine inter-hospital courier and portering services were used to transport specimens. There was no alteration to the routine specimen transportation systems during this study.

5.4.1c Laboratory processing

1. Swabs from the nose, axilla and groin were pooled for both IDI-MRSA™ and culture testing. All other specimens were processed individually.
2. If only one set of admission swabs/specimens was received during the intervention phase, culture was performed first, followed by IDI-MRSA™ (a method validated in-house).
3. Throughout the study, one of set of discharge swabs/specimens was taken and processed by conventional culture only.
4. The time of processing of IDI-MRSA™ tests mirrored the time of culture processing.
5. The routine diagnostics laboratory processed specimens until 7pm on weekdays and once a day on weekends.

6. IDI-MRSA™ was run in batches on specimens received by 9am, 12:30pm, 3:30 pm and 7pm. There was one IDI-MRSA™ run per day on weekends and bank holidays.
7. All IDI-MRSA™ and culture processing conformed with Clinical Pathology Accreditation (CPA) requirements and was subjected to a CPA inspection.
8. The culture method was the standard laboratory method used in the laboratories for routine MRSA screening, which is an MRSA selective broth (made in-house) (Gurran C *et al,*. 2002). This was coupled with Chromagar (Oxoid, Basingstoke, UK) and VITEK (bioMérieux, Basingstoke, UK) sensitivity testing from May 2006.
9. All culture screening for the trial was performed in the routine diagnostics laboratory as part of the routine diagnostic work.
10. A separate research team was employed to carry out IDI-MRSA™ testing.

5.4.1d Result reporting

1. Results were reported as soon as they were ready after verification according to laboratory standard operating procedures. However, the results from the 7pm IDI-MRSA™ run were not communicated to the wards until the following morning. Verification and reporting complied with CPA accreditation requirements and was subject to CPA inspection.
2. All results were reported electronically.
3. MRSA positive results were also be reported verbally to the relevant ward as soon as possible after they were ready.
4. Verbal reporting of MRSA positive results continued to be performed by the Trust infection control nurses and the Trust microbiology doctors (as per the standard Trust reporting procedures for routine MRSA positive results that were not a part of this study). During the intervention phase, both the culture and IDI-MRSA™ results were reported, irrespective of the availability of the paired result. Thus IDI-MRSA™ and culture discordant results were reported.
5. Patients with discordant IDI-MRSA™ and culture results were treated as MRSA positive.

6. All staff, including study investigators, were not blinded to the IDI-MRSA™ or culture results.

5.4.2 MRSA control

1. Policies and procedures for MRSA prevention and control were in accordance local policies which were based on national guidelines (Coia JE *et al*., 2006; Pratt RJ *et al*., 2001).
2. All in-patients were nursed with contact precautions for their hospital stay.
3. MRSA positive patients were additionally isolated in a side room where possible. If a side room was not available, MRSA positive patients were barrier nursed individually or in cohorts on the open ward.
4. MRSA positive patients were given decolonisation treatment including chlorhexidine gluconate washes (4% chlorhexidine gluconate. Instructions are to moisten skin and apply undiluted antiseptic particularly to known carriage sites such as the axilla, groin & perineum. Hair is also washed using 4% chlorhexidine gluconate) and topical chlorhexidine acetate (CX) powder. Washing continues for 5 days. Depending upon antimicrobials sensitivity data and contraindications, topical antimicrobial cream (usually 2% mupirocin or Naseptin (which contains chlorhexidine hydrochloride and neomycin sulphate) is applied to the nasal mucosa, three times daily for 5 days.
5. MRSA positive patients were treated as MRSA positive until they had had three MRSA negative specimens (by whichever method was positive) at least one week apart.
6. The electronic patient records and paper notes of all MRSA positive patients were permanently flagged with an 'MRSA History' label.
7. If possible, patients previously known to be MRSA positive or with other risk factors for MRSA carriage, were ideally isolated in a side room or barrier nursed on the open ward from the time of admission. This is known as 'pre-emptive isolation'. This was usually stopped if the admission screen was MRSA negative.
8. Patients identified as MRSA positive in pre-admission clinic were given decolonisation therapy in clinic and their surgical admission was postponed.

9. Patients with an MRSA positive result were given vancomycin or teicoplanin prophylaxis.
10. Patients who were MRSA positive by either method were re-screened for MRSA at weekly intervals and were considered to be 'MRSA negative' when they had had 3 negative screens (by whichever method was positive) at least 1 week apart.
11. MRSA negative patients are nursed with universal precautions for the duration of their stay.

5.4.3 Inclusion criteria

1. All patients admitted to the study wards who gave informed verbal consent for admission and discharge MRSA screening and who were MRSA negative on admission screening were potentially eligible for inclusion in the primary outcome analysis.

5.4.4 Exclusion criteria

Patients were excluded from the primary outcome analysis if:

1. They were transferred as 'MRSA positive' from another hospital (from here on referred to as 'MRSA positive on admission').
2. They were MRSA culture positive on any specimen taken up to five days prior to the current hospital admission (from here on referred to as 'MRSA positive on admission').
3. They were MRSA culture positive on any specimen taken during the same hospital admission, prior to study ward admission (from here on referred to as 'MRSA positive on admission').
4. They were MRSA culture positive on specimens taken within the first 48 hours of study ward admission, including the MRSA admission screen (from here on referred to as 'MRSA positive on admission').
5. Their admission screening swabs were taken > 48 hours after admission.
6. Their MRSA admission screen did not contain nose, axilla and groin swabs.
7. They were not swabbed on admission.

8. Their discharge screen did not contain nose, axilla and groin swabs
9. Their discharge screen did not contain nose, throat and perineum swabs and was taken on ITU. (Nose, throat and perineum are the sites routinely screened on ITU) The detection of MRSA from these sites is more sensitive than from nose, axilla and groin. This situation was applicable where patients were transferred to ITU and screened within 48 hours after discharge from the study ward).
10. They were not swabbed on discharge (from here on referred to as 'lost to follow up').

5.4.5 Withdrawal criteria

1. There were no withdrawal criteria. Patients could request to be withdrawn.

5.4.6 Regimen allocation

1. Professor CJM Whitty undertook the randomisation of wards by random number table on a different site (LSHTM). Wards were assigned numbers and randomised within blocks (surgical and medical randomised separately). Those wards randomised to the intervention arm started with the rapid test, those to the control arm started with conventional testing. All wards swapped over after a 1-month washout period so that previous controls became interventions and vice versa. Dr Dakshika Jeyaratnam, who conducted the study at GSTT, implemented the randomisation.

5.4.7 Adverse events

Potential adverse impact on patients in this trial was limited. A patient could be identified as MRSA positive by the IDI-MRSA™ test and not by culture, which might be a false positive result. Such patients might have unnecessary decontamination therapy, isolation and/or delay to scheduled surgery. Also, patients and staff who are contacts of a 'IDI-MRSA™ false positive' patient might undergo unnecessary screening and/or other investigations. However, it is known that standard culture screening may produce false-negative results so it is difficult to know which patients

are truly falsely identified as MRSA positive by IDI-MRSA™. Although it was appreciated that adverse events might occur as a consequence of false positive MRSA identification, this was not considered to be a serious event. Furthermore, MRSA detection by the IDI-MRSA™ test in a culture screen-negative patient might be beneficial. Nevertheless, the welfare of patients was monitored in this regard and action taken where necessary. All staff involved in the data collection and who visited the study ward each day attended a Good Clinical Practice for Clinical Trials course.

5.4.8 Interim analysis/Stopping rule/discontinuation criteria

Interim analyses were not conducted; this crossover design did not make an interim data analysis valid. The welfare of all patients during the study was monitored to ensure that no unforeseen adverse events arose (see section 3.8). A formal data and safety monitoring board (DSMB) was not constituted because there was no reasonable expectation they would be in a position to stop the trial based on interim data or adverse events. Serious ethical concerns that arose during the trial would be reported to the ethics committee chair. In cases of serious concerns the trial would be halted pending the decision of the ethics committee chair. There was no external review board. CONSORT criteria and the ORION statement were used to inform the study design and the reporting of results. (Moher D *et al*., 2001; Stone SP *et al*., 2001; Schulz KF *et al*., 2010).

5.4.9 Confounders

5.4.9a Patient confounders

Data on potential confounding factors was collected on all patients. The age, sex, length of study ward stay, American Society of Anaesthesiology (ASA) score (American Society of Anesthesiologists, 1963), and temporary transfers to other hospital areas were recorded.

5.4.9b Ward confounders

Potential ward confounding factors were collected monthly which were:

a) The compliance with hand decontamination policy. This was measured by unobtrusive direct observation using the Trust audit tool. Hand decontamination is a key infection control intervention required to interrupt the transmission of MRSA. It was calculated as the percentage compliance with hand decontamination before each patient contact.

b) The number of occupied bed days. This is a measure of ward and hospital activity. The greater is the level of activity, the greater is the risk of cross transmission and infection due to the increased density of patients on the ward and the greater demands on staff time. Higher bed occupancy make compliance with infection control measures more difficult due to demands on staff time.

c) Defined daily doses of antibiotics that are known to select for MRSA colonisation i.e. beta lactams & ciprofloxacin. Defined daily doses of antibiotics that are used to treat MRSA infections (vancomycin, teicoplanin and linezolid). An increased amount of the former will select for MRSA colonisation. An increased amount of the latter may reduce an individual's bacterial load of MRSA and subsequently decrease the chances of cross-transmission or the development of MRSA infection. The converse will happen with reduced amounts of these classes of antibiotics.

d) Staffing numbers, staff turnover & bank and agency (temporary) staff levels. Lower staffing levels make compliance with infection control measures more difficult due to demands on staff time. High staff turnover, temporary staff and short staffing are associated with reduced compliance with infection control practices. With temporary staff this is usually as a consequence of unfamiliarity with Trust procedures or due to failure to enrol on continuing professional development programmes. Staffing levels were calculated as a ratio of whole time equivalents to open beds. Agency and bank nurses were calculated in whole time equivalents.

e) The number of beds and side rooms open. The number of beds open on the ward is a reflection of the level of activity on the ward and the number of side rooms open is a direct measure of isolation facilities available.
f) The number of MRSA culture positive patients with isolation precautions.
g) The number of MRSA culture positive patients without isolation precautions.
h) The percentage of patients MRSA positive on admission that were isolated at admission.
i) MRSA colonisation pressure, defined as the proportion of patients who were MRSA positive on admission, was expressed as the number of patients who were 'MRSA positive on admission' (see 'Inclusion and Exclusion criteria' above for definition) per 1000 ward admissions. This is a measure of the burden of MRSA being brought on to the ward. These patients are the reservoir for spread of MRSA.

We noted the number of Trust infection control nurses. The proportion of time that MRSA positive patients were not isolated in a side room but cohort nursed on the open ward was used as a confounder. The number of patients who were MRSA culture positive (at any time, rapid test positive or negative) and barrier/cohort nursed on the open ward per day provided a measurement in patient-days. We performed the same calculation but for patients who were nursed without precautions on the open ward.

5.4.10 Other data collected

We recorded the location that the patient was discharged to as:

a) discharged for infection control reasons (which may not have been for the patient themselves)
b) discharged home/to another ward
c) deceased
d) ward closed
e) discharged to ITU
f) discharged to overnight intensive recovery (theatre area).

We noted the bed position, any infection control measures and the reasons for them for each patient between the time of admission to the study ward and the time of the admission screen result.

We recorded ward closures, bed closures and the reasons for them, the position of all patients with infection control precautions and the reason for them and the number of beds closed due to MRSA were noted daily.

5.4.11 Assessment of efficacy

5.4.11a Assessment of efficacy; primary outcome: MRSA Acquisition Rates

An MRSA acquisition was defined as a patient who becomes MRSA culture positive on MRSA screens or clinical specimens taken >48 hours after admission and ≤ 48 hours after discharge (the patient meeting inclusion criteria and not meeting exclusion or withdrawal criteria). This was expressed as a percentage of the patients recruited to the analysis.

There are multiple measures of MRSA acquisition in the medical literature. In order to compare our results with others, we also calculated the MRSA acquisition rate per 1000 patient-days at risk as well as the MRSA transmission rate. The patient-days at risk were the sum of the lengths of stay on the study wards for all patients recruited to the study in each arm. The MRSA transmission rate was defined as the ratio of patients who are MRSA positive on admission to the number of MRSA acquisitions.

5.4.11b Assessment of efficacy; secondary endpoints.

All analyses intention-to treat unless otherwise specified.

a) Clinical

i) An MRSA wound infection was identified by the presence of MRSA in a clinical wound swab specimen. Only one infection was considered per patient per admission. Clinical assessment of the wound, the patient's inflammatory markers or treatment of the MRSA isolate from the wound with antimicrobials were not used to identify infection.

ii) MRSA bacteraemias defined as the number of patients with MRSA positive blood cultures. Only one bacteraemia was considered per patient per admission.

b) Resource Use

iii) We calculated the number of days during which patients who were admission screen negative (by either method) were pre-emptively nursed with infection control precautions due to MRSA risk factors ('inappropriately barrier nursed')

iv) We calculated the number of days during which patients who were MRSA positive on admission (by either method) were nursed without MRSA infection control precautions from the time of study ward admission ('inappropriate open').

c) Test Specific outcomes

v) We calculated IDI-MRSA™ sensitivity, specificity, positive predictive and negative predictive values using the routine admission culture screen as the gold standard. We only included admission screens (AS) containing nose, axilla and groin swabs.

vi) MRSA screens which gave a discrepant result of culture negative/IDI-MRSA™ positive were investigated further. The screening swabs which had been tested by IDI-MRSA™ were stored at 4°C after processing and were available for further investigation, which happened as soon as possible after the discrepant result was known. The screens which had been processed by culture in the diagnostic laboratory were no longer available as they were discarded soon after processing. One ml of brain-heart infusion broth was added to the sample buffer tubes which still contained the swab tips. The swab tip remained in the broth and the tube was incubated aerobically for 18-24 hours at 35-37°C after which, a loop of the broth was plated on to Columbia blood agar (Oxoid, Basingstoke, UK) and re-incubated for 18-24 hours of aerobic incubation at 35-37°C. The plates were examined for 5 consecutive days for evidence of bacterial growth. Suspected *Staphylococcus aureus* isolates were confirmed by Gram stain and tested for catalase production, detection of the fibrinogen affinity antigen (clumping factor), protein A and the capsular polysaccharides of *S. aureus* using Pastorex® (Bio-Rad, Hemel Hempstead, U.K.), and mannitol fermentation. Susceptibilities to meticillin and ciprofloxacin were determined according to BSAC disc diffusion methods (Andrews JM *et al*., 2005).

vii) The turnaround time was defined as the time between admission to the study ward and the AS screening result that determined MRSA status. This was the first MRSA positive screen result by either method or the last MRSA negative screen result (meaning the last of all of the specimens, which formed a part of the MRSA admission screen).

5.4.12 Data analysis

All data was double entered in to Microsoft Access and analysed using Stata version 9.0 (StatCorp, College Station, Texas, USA). The final analytical plan was agreed prior to the analysis being undertaken. No changes to the primary and secondary endpoints were made from those in the protocol during the study or the analysis.

5.4.12a Primary analysis: MRSA acquisition rate

We calculated unadjusted odds ratios and then adjusted them in a generalized estimating equations (GEE) regression taking into account the cluster randomised design. A further, adjusted analysis was undertaken using the pre-defined potential confounding factors of age, sex, American Society of Anesthesiology (ASA) score on admission, ward and length of study ward stay. Standard errors were adjusted for within ward correlation. Odds ratios of regression coefficients were calculated.

Two groups of patients who met the inclusion criteria and appeared to have acquired MRSA on the wards, may have been MRSA positive on admission. In routine practice, patients who are MRSA positive on first screening specimens taken within 48 hours of admission are conventionally considered to have been admitted with MRSA. In the present primary analysis, patients with MRSA negative admission screens and MRSA positive discharge screens taken within 48 hours of admission were counted as MRSA acquisitions. It is possible that such patients had false negative admission swabs and did not acquired MRSA on the ward. Similarly, patients with a history of previous MRSA carriage in the three months prior to admission may have false-negative admission screens and subsequent positive discharge screens. We therefore performed a further restricted analysis.

For primary and major secondary outcomes patients who were not correctly swabbed on discharge were designated ‘lost to follow-up’ and were not included. The number falling into this category was reported.

5.4.12b Secondary analyses

For the analysis of resource use, patients who were MRSA positive on admission and those lost to follow up were additionally included in the analysis.

For the analysis of test specific outcomes all admission screen sets which contained nose, axilla and groin swabs were included.

5.4.13 Sample size calculation and study duration

With an estimated acquisition rate of 2% a halving of acquisitions to 1% required 3330 patients in both arms, accounting for the cluster design (α 0.5 β 90%). This study size will detect a reduction from 3% to 1.9% and from 4% to 2.7%. Reductions smaller than this were considered unlikely to trigger a change in practice given the resource implications.

5.5 Ethical issues

MRSA admission screening is routinely performed in many areas of Guy's and St. Thomas' NHS foundation Trust including the wards involved in this study. When these screens are performed, the member of staff taking the specimen obtains verbal informed consent. After application to our local COREC committee it was agreed that verbal informed consent should continue to be obtained for MRSA admission screens and also for discharge screens, the latter being introduced for this study.

5.6 Funding source

The funding source (The Department of Health) did not have any role in the study design, study execution, study analysis, the writing of study manuscript or the conclusions.

5.7 Trial registration

Clinical controlled trials ISRCTN75590122

Chapter 6: The rapid MRSA screening study results

The rapid MRSA screening study results

The study ran from January 2006 to March 2007 and comprised a three month baseline period, five month intervention period, one month washout period, and five month second intervention period. During the intervention periods 9608 patients were admitted to the study wards; 637 (6.6%) did not meet the inclusion criteria. Figure 1 shows the flow of participants through the study. Overall, 597 (6.7%) swabbed patients were culture positive for MRSA on admission (298 in the control arm, 299 in the rapid test arm). Of 8374 patients who met the study entry criteria, 1486 (17.8%) were lost to follow-up (18.6% in the control arm, 16.9% in the rapid test arm). In both arms 99% of the patients lost to follow-up were not swabbed on discharge, either because of an oversight by nursing staff or because the patients left the hospital before being swabbed. The remaining patients were swabbed but the samples at discharge were not from the correct anatomical sites. Thus 6888 patients had full data and were eligible, 3335 (81.4%) in the control arm and 3553 (83.1%) in the rapid test arm. The intervention, MRSA admission screening by IDI-MRSA™, was carried out in 4528 (99.0%) of 4783 patients who were admitted to the study wards and assessed for eligibility (Figure 1). Table 1 shows the baseline characteristics of the patients in both study arms, Table 2 the baseline characteristics of patients lost to follow up in both study arms and Table 3 the characteristics of the wards.

MRSA was acquired by 108 (3.2%) patients in the control arm and 99 (2.8%) in the intervention arm. The control and intervention arms did not differ for MRSA acquisition rate (unadjusted odds ratio 0.88, 95% confidence interval 0.52 to1.46, P=0.61), MRSA acquisition rate per 1000 patient days at risk (4.9 in the control arm, 4.4 in the rapid test arm; incidence rate ratio 0.90, 95% confidence interval 0.69 to 1.2, P=0.27) and transmission rate (0.36 in the control arm, 0.33 in the rapid test arm, incidence rate ratio 0.85, 0.64 to 1.12, P=0.24). This was unchanged when the acquisition rate was adjusted using generalised estimating equation regression for the predefined confounders (adjusted odds ratio 0.91, 95% confidence interval 0.61 to 1.34, P=0.63). At the rate of acquisition seen in the control arm, the study had the power to detect a reduction to 2%.

In the restricted analysis 17 patients in the control arm and 15 in the rapid test arm were excluded because they had a study ward stay of 48 hours or less (n=15) or they had been MRSA culture positive in the three months before admission (n=16); one patient had both. With these exclusions the adjusted odds ratio in generalised estimating equation regression was 0.86 (95% confidence interval 0.60 to 1.26, P=0.46).

MRSA wound infections occurred in 22 patients in the control arm and 21 patients in the rapid test arm (odds ratio 0.91, 0.48 to 1.7, P=0.77). Two MRSA bacteraemias occurred during the control phase and one during the intervention phase (0.49, 0.01 to 9.1).

MRSA was endemic on the study wards (table 4). Ward results varied but no systematic significant difference was found in MRSA acquisition or transmission rates between the intervention and control arms on individual wards, except during an MRSA outbreak that occurred on one ward during the control phase and another ward during the intervention phase.

A univariable analysis (table 5) showed that MRSA acquisition was associated with compliance with hand hygiene policy, the number of days that MRSA culture positive patients were cohort nursed on the open ward, and the number of days that MRSA culture positive patients were on the open ward but were not cohort nursed. When these potential independent factors were included in the generalised estimating equation regression model the adjusted odds ratio for MRSA acquisition was 0.85 (95% confidence interval 0.65 to 1.13, P=0.26).

The control and intervention arms differed significantly in the number of inappropriately isolated or cohorted days (399 v 277, respectively, P<0.001). In the control arm 303 days inappropriately isolated or cohorted (75.9%) and in the intervention arm 221 such days (79.8%) were spent in side rooms. The proportion of patients who were pre-emptively isolated or cohort nursed was similar between the two arms (5% in the control arm, 4.7% in the rapid test arm). A small, statistically insignificant difference was found for the number of inappropriate open days between the two arms (389 in the control arm v 351 in the rapid test arm, P=0.08). However,

this result included patients who were MRSA positive by both culture and IDI-MRSA™, as well as patients who were MRSA positive by IDI-MRSA™ only. The latter group required isolation but were unique to the intervention arm. This may explain why the difference between the two arms was small and insignificant. By using culture results only and by excluding IDI-MRSA™ results from the analysis we were able to compare like with like between the control and intervention arms. The difference in the number of inappropriate open days between the two arms was then statistically significant (389 in the control arm v 213 in the rapid test arm, P<0.001). Fifteen (0.22%) of the 6888 patient were present in more than one phase of the study due to a long length of stay which included the wash-out period; they were not re-screened.

Four of 4558 (0.09%) tests on admission samples did not produce a result owing to inhibition of the polymerase chain reaction. For the remaining specimens the sensitivity of the rapid test compared with conventional culture was 87.8% and the specificity was 96.3% (positive predictive value 55.1%, negative predictive value 99.4%; table 6).

The median (interquartile range) turnaround time from admission, including portering, processing, and reporting, was 46.4 hours (39.1-66.1) for conventional culture in the control phase and 21.8 hours (17.9-25.4) for the rapid test (P<0.001). The time between a positive result being available electronically and being telephoned to the ward during the rapid phase was calculated for 260 MRSA positive patients; four (1.5%) were telephoned the day before the computer result, 217 (83%) the same day, 31 (11%) the day after, six (2.3%) two days after, and one (0.38%) each three and four days after.

Seven of the included patients who were MRSA culture negative on admission and MRSA culture positive by discharge were positive on admission using the Polymerase chain reaction test; these cases were counted as MRSA acquisitions by study definitions. When these patients were excluded from the analysis the difference in MRSA acquisition between the two arms remained statistically insignificant (P=0.13).

An outbreak of MRSA occurred in one ward during the intervention phase (closed for six days) and another ward during the control phase (closed for five days). Because of diarrhoea and vomiting outbreaks, one study ward closed for eight days (intervention phase), another for 11 days (control phase), and another for one day (intervention phase). One ward closed permanently in November 2006 (control phase) and a similar ward was recruited. Since this could have affected results a restricted analysis was undertaken with the closed ward and recruited ward removed; this made little difference to the MRSA acquisition rate (adjusted odds ratio 0.90, 95% confidence interval 0.65 to 1.24, P=0.52). One of the study wards moved to the location of the closed ward in February 2007 (control phase). A further ward had some refurbishment and cleaning over nine days (intervention phase).

158 of 159 screening tests which gave a culture negative/IDI-MRSA™ positive result (one set of sample buffer tubes could not be found) were tested in order to identify isolates of *Staphylococcus aureus* and if found, determine their susceptibility to meticillin and ciprofloxacin. The majority of specimens were mixed with a variety of organisms of which thirty-seven (23.4%) yielded MSSA, MRSA was isolated from 29 (18.2%) and both MRSA and MSSA were isolated from 2 (1.2%). Therefore, in total 31 (19.6%) of the culture negative/IDI-MRSA™ positive specimens yielded MRSA. Of the 31 MRSA isolates, 21 (67.7%) were ciprofloxacin susceptible and (10) 32.3% were resistant. Ciprofloxacin susceptibility testing was performed for 34 of the 39 MSSA isolates, of which 31 (91.2%) were ciprofloxacin susceptible. The remaining 91 tests (57.6%) grew a mixture of organisms including coagulase negative Staphylococci, *Streptococcus* spp., *Bacillus* spp., *Enterococcus* spp. and coliforms. These were not investigated further.

Figure 1 Flow of patients through the study

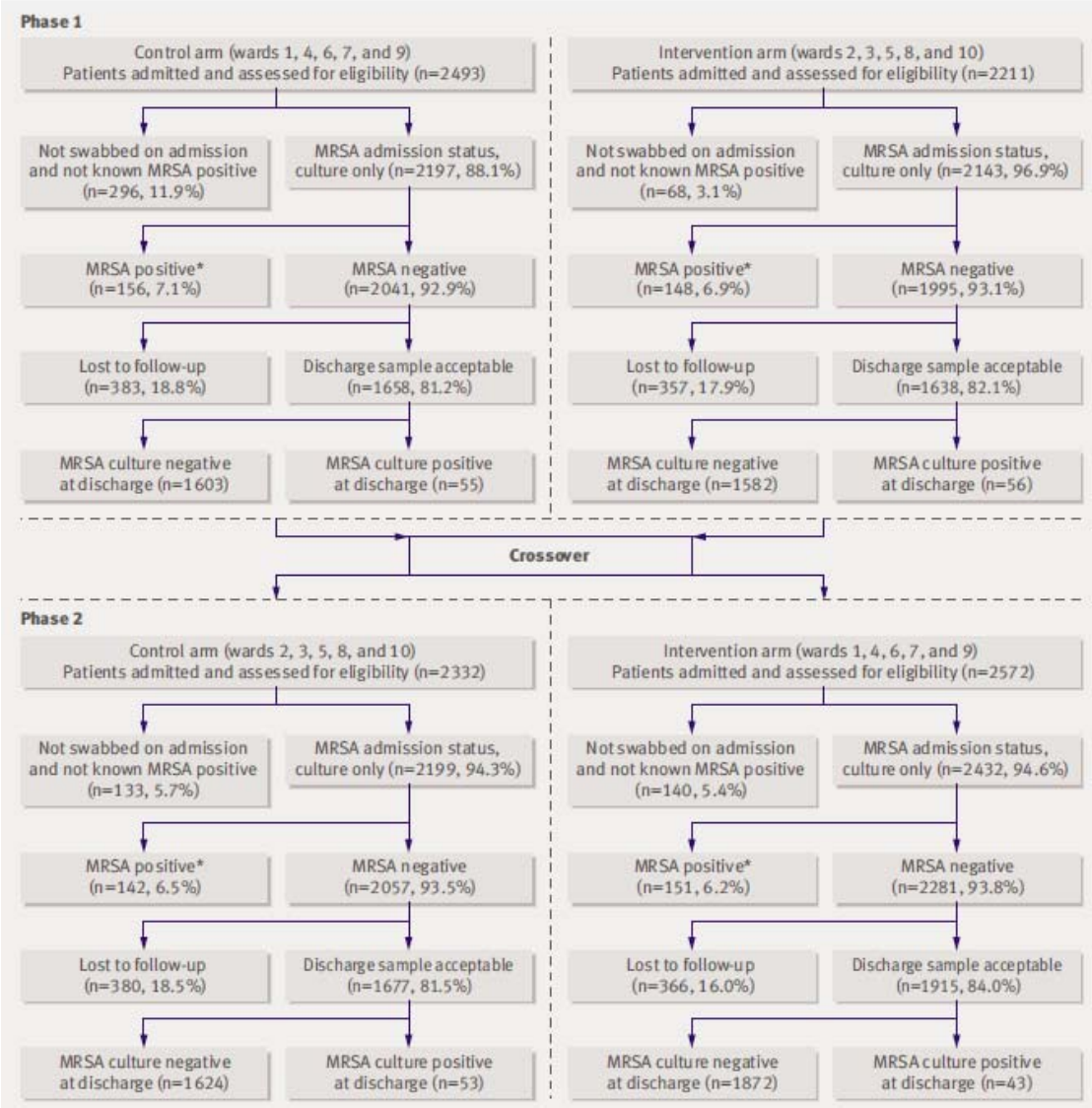


Table 1 Baseline characteristics of patients in crossover study of rapid polymerase reaction test compared with conventional culture (control group) for detection of methicillin resistant *Staphylococcus aureus*. Values are numbers (percentages) unless stated otherwise

Characteristics	Control phase	Intervention phase
Median (interquartile range) age (years)	59.1 (40.5-72.7)	58.5 (41.2-71.6)
Women	1436 (43.1)	1412 (39.7)
Median (interquartile range) ASA score*	2 (2-3)	2 (2-3)
Patients who visited temporary locations	81 (2.4)	92 (2.6)
Median (interquartile range) stay on study ward (days)	3.3 (1.8-7.2)	3.8 (1.7-6.9)
No of patient days at risk	22 018	22 275
MRSA culture positive on admission	298 (6.8)	299 (6.5)
MRSA culture positive on admission but pre-emptively isolated before positive result	102 (34)	93 (31)
Median (interquartile range) study ward stay for patients MRSA culture positive on admission	7.1 (3.1-13.1)	6.5 (3.1-15.0)
Mean No of MRSA screens sent per patient	2.0	2.1

Data for all patients included in analysis of primary and major secondary outcomes (except data for patients MRSA culture positive on admission).

*American Society of Anesthesiology score for physical status. 1 = a completely healthy patient, 2 = a patient with mild systemic disease, 3 = a patient with severe systemic disease that is not incapacitating, 4 = a patient with incapacitating disease that is a constant threat to life, 5 = patient is moribund, not expected to live 24 hours

Table 2 Baseline characteristics of patients lost to follow up in crossover study of rapid polymerase reaction test compared with conventional culture (control group) for detection of methicillin resistant *Staphylococcus aureus*. Values are numbers (percentages) unless stated otherwise

Characteristics	**Control phase**	**Intervention phase**
Median (interquartile range) age (years)	58.1 (41.3-74.5)	60.4 (41.2-74.9)
Women	362 (47.4)	339 (46.9)
Median (interquartile range) ASA score*	2 (2-3)	2 (2-3)
Patients who visited temporary locations	11 (1.4)	9 (1.3)
Median (interquartile range) stay on study ward (days)	3.6 (1.4-7.8)	3.1 (1.3-6.8)
No of patient days at risk	4777	4822

*American Society of Anesthesiology score for physical status. 1 = a completely healthy patient, 2 = a patient with mild systemic disease, 3 = a patient with severe systemic disease that is not incapacitating, 4 = a patient with incapacitating disease that is a constant threat to life, 5 = patient is moribund, not expected to live 24 hours

Table 3 Characteristics of wards in crossover study of a rapid polymerase reaction test compared with conventional culture for detection of methicillin resistant *Staphylococcus aureus*

Ward No, specialty	**Hospital site**	**No of bays and bed capacity**	**No of side rooms**	**No of beds**
1, surgery (plastics)	St Thomas'	2 bays with 6 beds, 3 bays with 4 beds	4	28
2, elderly care	St Thomas'	2 bays with 6 beds, 3 bays with 4 beds	4	28
3, surgery (urology)	Guy's	3 bays with 6 beds, 1 bay with 5 beds	3	26
4, surgery (ear, nose, and throat)	Guy's	4 bays with 6 beds, 1 bay with 4 beds	6 (5 rooms with 1 bed, 1 room with 2 beds)	35
5, surgery (cardiothoracic)	Guy's	1 bay with 15 beds, 1 bay with 14 beds	3	32
6, elderly care	St Thomas'	2 bays with 6 beds, 3 bays with 4 beds	4	28
7, surgery (vascular	St Thomas'	2 bays with 6 beds, 3 bays with 4 beds	4	28
8m surgery (gastrointestinal)	St Thomas'	2 bays with 6 beds, 3 bays with 4 beds	4	28
9, oncology	Guy's	1 bay with 9 beds, 1 bay with 4 beds	12	25
10, oncology	Guy's	2 bays with 12 beds	4	28
11, oncology	Guy's	2 bays with 10 beds	4	24

Wards 2, 3, 5, 8, and 10 were randomised to receive the intervention first.

Table 4 Results from wards using conventional culture (control group) or a rapid polymerase chain reaction test to detect meticillin resistant *Staphylococcus aureus* (MRSA)

Ward No	Importation pressure*		Odds ratio (95% CI) for MRSA acquisition rate	P value	MRSA transmission rate		MRSA acquisition per 1000 patient days	
	Control group (%)	Rapid test group (%)			Control group	Rapid test group	Control group	Rapid test group
1	6.8	3.7	0.59 (0.13 to 2.64)	0.49	0.12	0.14	1.88	1.33
2	15.5	19.0	2.61 (0.24 to 5.52)†	0.01	0.36	0.58†	4.69	9.43†
3	7.0	6.1	1.20 (0.57 to 2.54)	0.63	0.25	0.35	5.89	7.61
4	4.8	5.1	1.00 (0.47 to 2.1)	0.99	0.33	0.32	5.17	5.29
5	3.6	2.7	0.50 (0.15 to 1.67)	0.26	0.42	0.29	3.71	1.87
6	19.7	14.8	0.31 (0.15 to 0.67)†	<0.01	0.65†	0.34	10.25†	5.92
7	7.9	8.4	1.14 (0.47 to 2.77)	0.78	0.27	0.28	3.94	4.66
8	5.1	6.8	0.51 (0.21 to 1.27)	0.15	0.68	0.25	6.26	2.84
9	5.0	4.3	0.39 (0.10 to 1.56)	0.18	0.50	0.25	2.96	1.28
10/11	6.0	9.2	1.60 (0.50 to 5.07)	0.43	0.30	0.32	3.99	4.02

*Proportion of patients positive for MRSA on admission.

†Outbreak of MRSA.

Table 5 Potential confounding factors tested for association with outcome

Variables	Median (interquartile range) per month	Univariable analysis odds ratio (95% CI)	P value
β lactam antibiotics*	659.75 (316.57-1354.03)	0.807 (0.614 to 1.060)	0.123
Ciprofloxacin*	214.30 (127.50-542.50)	1.000 (0.392 to 2.532)	0.993
Anti-MRSA antibiotics*	15.01 (7.93-26.71)	0.594 (0.010 to 35.079)	0.802
Occupied bed days	654 (568-746)	4.66 (0.20 to 111.19)	0.342
Importation pressure† (per 1000 admissions)	53 (36.75-95.75)	1.002 (0.998 to 1.006)	0.326
Hand washing compliance (%)	41.7 (20.0-51.58)	1.080 (1.028 to 1.134)	0.002‡
Patients days§	2 (0-10.00)	1.113 (0.979 to 1.265)	0.101‡
Patients days¶	0 (0-1)	1.055 (1.020 to 1.091)	0.002‡
MRSA positive on admission but isolated (any reason) before positive result (%)	33 (16.5-50)	0.995 (0.986 to 1.004)	0.264
Staff turnover	0 (0-2.44)	1.041 (0.986 to 1.099)	0.144
Staffing levels (whole time equivalents/open bed)	0.79 (0.67-0.93)	0.235 (0.438 to 1. 263)	0.092
No of ward beds open(not side room)	28 (26-28)	0.974 (0.879 to 1.079)	0.612
No of side rooms open	4 (4-4)	1.301 (0.783 to 2.163)	0.310
Use of bank and agency staff (whole time equivalents)	2.9 (1.69-5.37)	0.945 (0.840 to 1.062)	0.341

MRSA=meticillin resistant *Staphylococcus aureus*.

*Defined daily doses. WHO standardised measure of drug consumption

†Proportion of patients MRSA positive on admission.

‡Included in multivariable analysis.

§MRSA culture positive patients cohort nursed on open ward.

¶MRSA culture positive patients on open ward but not cohort nursed.

Table 6 Characteristics of rapid polymerase chain reaction test for meticillin resistant *Staphylococcus aureus* (MRSA) in samples taken on admission

Variable	MRSA culture result		No of admission samples
	Positive	Negative	
Rapid test result:			
Positive	195 (87.8)*	159	354
Negative	27	4173 (96.3)*	4200
No of admission samples	222	4332	4554

*Number (percentage) of samples.

Chapter 7: The rapid MRSA screening study discussion

The rapid MRSA screening study discussion

The control of Meticillin resistant *Staphylococcus aureus* cross transmission and infection is a global and national priority. It is widely accepted that targeting control measures on patients identified as MRSA positive at admission will facilitate the reduction of MRSA transmission in hospital wards. It is further hypothesised the earlier this identification is done the more effective control will be. Standard culture screens for MRSA take 2 days. The rapid identification of MRSA carriers within 24 hours of hospital admission is achievable with PCR-based tests and seems a logical addition to current control measures. This randomised controlled trial investigated the use of a rapid, PCR-based test, IDI-MRSA™ in the control of MRSA acquisition and cross transmission on hospital general wards, in comparison with conventional culture screens.

This study found that under operational conditions rapid PCR screening for MRSA reduced the time taken between ward admission and the MRSA admission screen result from 46.4 hours to 21.8 hours and had an impact on patient isolation and cohort nursing. However, it found no evidence of a significant reduction of rates of MRSA acquisition (3.2% versus 2.8%, p=0.61), MRSA transmission or MRSA infection when compared to conventional culture.

Data was collected contemporaneously on potential confounding factors. These included measures of antibiotic use, resources such as staffing and the number of side rooms available, compliance with infection control procedures such as hand decontamination, and measures of the MRSA burden such as the MRSA colonisation pressure. In a univariable analysis hand hygiene compliance, the number of patient days that MRSA culture positive patients were cohort nursed on the open ward, and the number of patient days that MRSA culture positive patients were on the open ward without any contact precautions, were associated with MRSA acquisition. However, when these factors were included in the generalised estimating equation regression model multivariate analysis, they were not associated with the outcome (adjusted odds ratio 0.85, 95% confidence interval 0.65 to 1.13, p=0.26). Therefore

these factors did not explain the lack of a difference between the intervention and the control.

Restricted analyses were performed that excluded patients with a hospital stay of ≤48 hours, those who had been MRSA positive within the last three months or both (overall 17 in the control arm, 15 in the intervention arm). Seven patients who were MRSA positive only by PCR on admission and who were culture positive on discharge swabs were also removed. This made no significant difference to the findings.

The wards in this study were chosen to be representative of the settings where MRSA transmission occurs in the UK.

There is no evidence that the staff in this study responded less well to a positive MRSA result than other hospitals in the UK. The majority of MRSA cases were isolated in side rooms. Importation pressure was similar to another, local hospital (Rao GG *et al.*, 2007) and to centres that have reported a reduction of MRSA rates with rapid MRSA screening (Cunningham R *et al.*, 2007; Jenks P *et al.*, 2007; Harbarth S *et al.*, 2006; Keshtgar MR *et al*., 2008). Hand hygiene rates, which were low and in keeping with covert observation, were similar to or better than those found at other UK hospitals (Cepeda J *et al.*, 2005; MacDonald A *et al.*, 2004). Robust data on MRSA acquisition and transmission rates is lacking from these other studies (Ritchie K *et al.*, 2006; Cooper BS *et al.*, 2004) and as these measurements are dependent upon ward case mix, comparison is difficult. Our rates are in keeping with good, non-outbreak studies that include general wards (Ritchie K *et al.*, 2006; Rioux C *et al.*, 2007) and the data does not suggest unusually poor MRSA control. The outbreaks and ward closures seen during this study are not untypical for hospital practice elsewhere in the UK. Whilst no two settings are identical, there is no obvious reason to believe that the findings in this large, randomised study cannot be generalised to other settings.

Our results are contrary to theoretical expectations (Bootsma MCJ *et al.,* 2006; Cooper BS *et al*., 2004; Raboud J *et al*., 2005; Kluytmans J, 2007). To date there are

ten other published studies that have investigated the impact of rapid MRSA screening on MRSA rates in clinical settings (Harbarth S *et al*., 2006; Conterno LO *et al*., 2007; Cunningham R *et al*., 2007; Harbarth S *et al*., 2008; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008; Aldeyab MA *et al*., 2009; Richer SL & Wenig BL, 2009; Hardy K *et al*., 2010). Though the studies have varying designs and results, all of them reported a significant reduction in the turn-around time of the MRSA screening tests during the intervention arm when PCR was used in comparison to the control arm. Of the ten studies, seven found that rapid MRSA screening was associated with a significant reduction in some or all of the MRSA rates that were measured (Harbarth S *et al*., 2006; Cunningham R *et al*., 2007; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008; Richer SL & Wenig BL, 2009; Hardy K *et al*., 2010). Three studies found that rates did not change significantly when rapid screening was used (Conterno LO *et al*., 2007; Harbarth S *et al*., 2008; Aldeyab MA *et al*., 2009) though for one study, this was due to an outbreak of MRSA during the PCR phase (Aldeyab MA *et al*., 2009).

The study presented in this thesis has some limitations. Firstly, we cannot eliminate a small effect of the PCR test on MRSA rates; a trial of over 60,000 patients would be needed to detect the size of difference found here. However given the cost of the test, rapid screening would be difficult to justify at this level of difference.

Secondly, neither the study investigators nor the study ward staff nor the patients were blinded to the method of MRSA detection. This was unavoidable because the turn-around time of the MRSA screening result was evident to all of these individuals and therefore, the method of detection being used was obvious. In order to compensate for this we measured confounding variables such as hand hygiene, which may have increased or decreased if the hospital staff knew which phase they were in.

Another flaw is that the method of MRSA detection in the control arm was a selective broth that contained ciprofloxacin. The advantages of such a broth include cost, cost-efficiency by allowing pooling of specimens, improved sensitivity by enrichment, it is easy to use and it has a relatively fast turn-around time in comparison to other culture methods. However, as the broth contains ciprofloxacin as a selective agent it should not detect ciprofloxacin sensitive organisms such as some of the recently described

strains of CA-MRSA (Health Protection Agency, 2005; Health Protection Agency, 2006). Failure to detect these strains during the study will have affected the control of MRSA on the wards and it would also have affected the performance characteristics of the PCR test. Our analysis shows that 21 (13.3%) of 158 culture negative/ IDI-MRSA™ positive tests yielded ciprofloxacin sensitive MRSA strains and 10 (6.3%) of the 158 tests contained ciprofloxacin resistant MRSA strains. However, throughout the study, all patients with a positive result by either method, even if the results were discordant were considered to be MRSA positive and had control measures in place. Therefore, it is unlikely that these patients contributed to the on-going transmission of MRSA in the intervention arm. Some carriers of MRSA may have been missed in the control arm but this would not explain the lack of a positive effect when IDI-MRSA™ testing was in place. In addition, clinical specimens are not processed using the selective broth. They are cultured on non-selective media such as sheep blood agar. An analysis at GSTT of the proportion of MRSA isolates from clinical specimens which were ciprofloxacin susceptible reported similar findings to those in the present study for the same year (13.6% in 2006) (Otter JA & French GL. 2008b). Therefore it is unlikely that poor detection of ciprofloxacin sensitive MRSA strains had an impact on the study outcomes.

Another criticism is that there were outbreaks of MRSA, and diarrhoea and vomiting on the wards during the course of the study. The largest outbreak of MRSA affected wards 2 and 6 at the same time. This was while one of these wards was in the control arm and the other was in the intervention arm. Any bias created by this outbreak was statistically ‘evened-out’ across the two arms. Outbreaks of diarrhoea and vomiting are well described in hospitals and reflect the real life setting of this study.

A further limitation is that approximately 20% of the patients in each arm were lost to follow up. It is possible that a greater number of patients who had acquired MRSA during their admission were lost to follow up in one arm of the study than in the other arm. This would affect the results. However, our analysis shows that the demographics associated with MRSA carriage for the patients lost to follow up are the same in both arms and so a biased result is unlikely.

Many of the studies on PCR MRSA screening published by others have been carried out in the intensive care unit (ICU) setting, where MRSA prevalence is often highest. One flaw of the present study is that it cannot be extrapolated to the ICU and it is therefore difficult to compare all of our findings against those found in ICU based studies.

The choice of the 'gold standard' is also debatable; we used culture as the reference. Others have investigated the BD GeneOhm™ system (formerly IDI-MRSA™), the Cepheid GeneXpert MRSA and broth-enriched culture (Hombach M *et al*., 2010). The sensitivity, specificity, and negative predictive value (NPV) were high for the BD GeneOhm™ system (100%, 98.5%, and 100%, respectively) and the Xpert MRSA (100%, 98.2%, and 100%, respectively). This group used MRSA results from other specimens taken from other anatomical sites to resolve the MRSA status of five patients with culture negative/PCR positive tests. By doing this the PPV of the BD GeneOhm™ test increased from 82.4%-87.5% to 93.3-94.1% and for the Cepheid GeneXpert from 66.7-92.9% to 88.9%-92.9%. Because there were no false-negative PCR results detected and due to the improved PPV of the tests once PCR positive/culture negative discrepant results were resolved, they concluded that PCR tests should be the gold standard. In this trial, the rapid test was under investigation and standard methods routinely used in real-life hospital practice were used in the control arm. We also found the IDI-MRSA™ test to have good performance characteristics with respect to sensitivity (87.8%), specificity (96.3%) and NPV (99.4%). However the PPV was 55.1%. We did not investigate further the 27 (0.6% of all tests) culture positive/IDI-MRSA™ negative MRSA strains. However we have shown that 20% of culture negative/IDI-MRSA™ positive tests grew MRSA when we re-cultured the specimens. It should be noted that the remaining 80% of these tests did not yield MRSA but instead a mixture of coagulase negative staphylococci, meticillin sensitive *Staphylococcus aureus* and no Staphylococci at all, suggesting that these tests were true false positive PCR tests. Such findings confirm that in this study, the culture method should be the reference standard.

Finally, we did not perform any assessment of the contribution of the environment to MRSA transmission, such as audits of near patient equipment, linen and waste handling, and environmental cleaning. It is possible that these surfaces will have

allowed the on-going cross transmission of MRSA and that contributed to the result. The role of the environment in the transmission of MRSA is uncertain though contamination of the inanimate surfaces around MRSA positive patients has been studied and quantified (Rohr U *et al*., 2009). It is known that cleaning methods and the frequency of cleaning can vary within and between wards and between institutions. In the present study the cross-over design should account for such variations within wards. We did not compare different wards with one another. For completeness, these are audits that we could have factored in to the study.

On the study wards at GSTT, MRSA positive patients are either isolated or cohort nursed on the open ward and given decontamination treatment, following national guidelines. This rigorous study provides no evidence that deploying universal rapid testing would improve usefully on universal culture testing to reduce MRSA.

7.1 Discussion of study findings

The study presented in this thesis found that using the IDI-MRSA™ PCR test for MRSA admission screening did not significantly reduce MRSA acquisition or transmission rates or MRSA bacteraemia or wound infection rates.

Of the published studies that have investigated rapid MRSA testing, seven have found a reduction in the incidence of MRSA and four have found that these tests had no such effect. However these studies differ from each other in a number of ways. These include the study design, collection of confounder data, the ward setting & the types of patients who were screened for MRSA, the anatomical sites screened for MRSA, the method of MRSA detection both with respect to the PCR test and the culture method, the prevalence of MRSA, whether or not MRSA decolonisation therapy was used, the isolation protocol and the inclusion and exclusion criteria for patients. This variation reflects the differences across the UK and worldwide with respect to MRSA control. The comparator in the eleven studies was either no MRSA screening or screening using standard culture techniques. During all phases MRSA control procedures were applied to MRSA positive patients, the nature of which varied between studies but all groups applied contact precautions with isolation ideally in a

side room. The outcomes measured include MRSA acquisition and transmission rates, MRSA surgical site infection (SSI) rates and MRSA bacteraemia rates.

7.1.1 Study design

The gold standard in interventional studies is a double blinded, randomised controlled trial. The present study is the only published one with a randomised, controlled trial design. The majority of the other published studies have an interventional cohort design with historical controls, which the authors admit is flawed (Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008). Studies with a cross over design (Harbarth S *et al*., 2008; Aldeyab MA *et al*., 2009; Hardy K *et al*., 2010) make the control and intervention groups more comparable than those without a contemporaneous control group. Of these, one found a positive impact of the rapid detection of MRSA (Hardy K *et al*., 2010), one found no improvement in MRSA rates (Harbarth S *et al*., 2008) and one was affected by an outbreak of MRSA (Aldeyab MA *et al*., 2009). It is always the case in studies with a historical control group (Harbarth S *et al*., 2006; Conterno LO *et al*., 2007; Cunningham R *et al*., 2007; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008; Richer SL & Wenig BL, 2009) and sometimes in prospective studies that confounders cannot be collected or that they are incompletely collected. Without these measurements it is difficult to assess whether or not the intervention was solely responsible for the results found in the study. One uncontrolled study found a significant reduction of *Staphylococcus aureus* bacteraemia, and to a lesser extent MRSA wound infections, when PCR screening was used relative to a historical control period during which there was no MRSA screening (Keshtgar MR *et al*., 2008). However, the authors state that in the four months immediately prior to the start of the PCR screening, patients had been moved to a new hospital building, which coincided with an increase in MRSA infections. With this move, a multitude of factors related to MRSA control would have changed. The authors attempted to compensate for this by using data from the six years prior to the move as the control data. However, the impact on the results of the recent move to a new building is not known and thus it is difficult to draw clear conclusions about MRSA control and PCR testing from this study.

7.1.1a Confounder data

7.1.1a (i) Measurable data

The study presented in this thesis measured a number of different confounders and these were included in a univariable and multivariable analysis. Others have measured some confounder data such as one study on a UK ICU, which included the proportion of patients who were successfully decolonised in the control and intervention arms (Cunningham R *et al*., 2007). This was similar: 36% in the control period and 33% in the PCR phase. This was the only confounder that was measured though the authors make the broad statement that there were no changes to infection control practices throughout the study. The present piece of work measured confounder data throughout the study period in both the intervention and the control arms. In another study, the only confounder assessed was compliance with isolation procedures and this was only observed during phase 3 of a three phase study (Robiscek A *et al*., 2008). These authors do not state whether or not this was done surreptitiously, but by observing isolation only during this phase, it is possible that the authors influenced the likelihood of whether or not a patient was isolated by way of the Hawthorne effect. Aldeyab *et al* found that rapid testing for MRSA did not reduce the incidence of MRSA on their surgical wards (Aldeyab MA *et al*., 2009). However during the intervention phase there was an outbreak of MRSA on these wards. The authors had audited compliance with infection control procedures throughout the study and these revealed a marked difference between the two phases with poor compliance with hand hygiene during the intervention phase on the surgical wards. The authors postulate that the reasons for the lack of an effect of the PCR test on these wards was that infection control practices were poor during the PCR phase. They recognised the importance of collecting such data and suggested that more audits were needed during their study.

7.1.1a (ii) Non-measurable data

There are actions that may affect a study's outcomes, which are non-measurable. These include the reporting of MRSA results to ward staff. For example, in a study on a UK ICU, which found that PCR testing reduced MRSA rates, during both intervention and control phases MRSA positive results were reported verbally either

by an infection control nurse or a consultant microbiologist. During the control phase an electronic report was issued on the same day as the telephoned result, but during the intervention phase, the IDI-MRSA™ was reported as provisional pending the culture result. There was a dedicated link nurse on the ICU who communicated with the infection control team and laboratory throughout the study (Cunningham R *et al*., 2007). Though the reporting mechanisms were similar between the two phases, problems arise. It is known that the behaviour of a reporter who is not blinded to the intervention may vary between the two phases. This may introduce some bias and no steps were taken by the authors to provide data to show that bias was probably not introduced, such as reporting the turn-around time to verbal reporting of results for both phases or compliance with infection control measures for MRSA positive patients. In contrast, in the present study turn-around times and infection control compliance were measured.

7.1.1a (iii) Multiple Interventions

In the present study, pre-emptive isolation, contact precautions and MRSA decolonisation therapy were already hospital MRSA control policy before the trial. PCR testing was a new addition to those control measures. Some other published studies introduce multiple interventions simultaneously. Deciphering the effect of each intervention on outcome in these cases can be very difficult. One example is a study in three affiliated hospitals (one teaching, one primary care and one community) in the USA, which had three consecutive intervention periods. These were: a baseline period (I) without an MRSA admission screening programme, followed by a second intervention period (II) when MRSA admission screening was introduced on the Intensive Care Units (ICUs) (ICU surveillance) and a final intervention period (III) when universal MRSA admission screening was introduced for all admissions (universal surveillance) (Robiscek A *et al*., 2008). In addition, during phase III, nursing staff were informed of results by telephone, decolonisation therapy was promoted and there were active feedback and education programmes in order to improve adherence with the screening programme. Swabs from the anterior nares were sent for testing by an in house PCR method in the second intervention period and this was replaced by IDI-MRSA™ in phase III. The primary outcome measure was the hospital associated MRSA infection rate, which included infections occurring 30 days after discharge. Secondary outcomes were the rates of MRSA bacteraemia,

MRSA infection rates up to 180 days after discharge and adherence to the MRSA surveillance programme. Rates of MSSA bacteraemia were used as a control. The two PCR tests were reported to have comparable performance characteristics. The turn-around time during the ICU surveillance phase was 2.5 days and 0.67 days during the universal surveillance phase. Screening adherence was initially 75% during the ICU surveillance period and increased to 90% by the end of period III. It was 84.4% during the universal surveillance period. During the ICU surveillance phase the prevalence of MRSA carriage on admission was 8.3% and during the universal surveillance phase it was 6.3%. The prevalence density of hospital acquired MRSA infections per 10 000 patient days was 8.9 during the baseline period (95% confidence interval 7.6 – 10.4), 7.4 during the ICU surveillance period (95% confidence interval 6.1 – 9.0) and 3.9 during the universal surveillance period (95% confidence interval 3.2 – 4.7). The reduction between the baseline and the period when universal screening, decontamination therapy and active education programmes were introduced was –5.0 per 10 000 patient days (95% confidence interval -6.6 to -3.5). This effect extended to rates 30 days post discharge but not at 180 days post discharge. MSSA bacteraemia rates did not change. The authors report that that the reduction persisted for 21 months, that there was no concurrent fall in MSSA bacteraemia rates and that patients had similar baseline characteristics during all three phases. However they concede that the introduction of universal surveillance may not have been responsible for the fall in MRSA infection rates. It is notable that decolonisation therapy was not given to patients who were MRSA positive on clinical specimens during phases I and II, or routinely during the ICU surveillance period, but it was recommended for all MRSA positive patients in the universal surveillance period. This treatment will have had an effect on MRSA control additional to the contact isolation that was in place during all three phases (Boyce JM, 2001). It should also be remembered that not all patients would have been placed in a side room (data not provided). It is possible, therefore, that if decolonisation therapy had been given to patients who were identified as MRSA positive either on culture alone during phase I or II and also to patients identified by screening during the ICU phase, that MRSA rates would have fallen. The individual effects of universal screening, ICU screening and decolonisation therapy cannot be deciphered. Further, the active feedback and education programmes in phase III may have improved compliance with infection control procedures such as hand hygiene. It is feasible that this intervention alone was responsible for the

difference measured in MRSA infection rates between the baseline period and period III. As variables such as hand hygiene compliance were not measured during the study it is not possible to make any assessment of their effect.

Another study on two ICUs introduced two interventions simultaneously (Harbarth S *et al*., 2006). These were universal rapid MRSA screening using an in-house PCR assay (qMRSA) and pre-emptive isolation. However, because the timing of the introduction of these two interventions differed between the two ICUs, it is possible to draw conclusions about the contribution of each to the control of MRSA. This study is discussed further in section 7.1.8.

7.1.2 Ward setting & the types of patients who were screened for MRSA

7.1.2a Intensive Care Unit

Patients may be at a greater risk of acquiring MRSA on the ICU than on general wards due to a greater prevalence of MRSA on such units, the higher number of health care workers to patient contacts, the use of a larger number of devices, including intravenous catheters and endotracheal tubes, and the greater use of antibiotics which may select for MRSA. It is also possible that isolation in a side room is not required in order to prevent cross transmission on the ICU (Cepeda J *et al.*, 2005). This may be due to the 1:1 nursing care that predominates throughout the working day. This is thought to be in contrast to the situation on general wards, though this has not been rigorously investigated.

Three published studies on PCR screening for MRSA were performed purely in an ICU setting. One was in the UK, one was in Switzerland and one was in the USA. Two of the studies on ICUs found that rapid testing for MRSA reduced the incidence of MRSA on these units. The first was a UK study on a mixed medical and surgical ICU, which used historical controls (Cunningham R *et al*., 2007). The main outcome measures were ICU acquired MRSA infections and the MRSA transmission rate. The overall prevalence of MRSA carriage on admission was 7.0%, which is similar to that found in the present study. During the control phase the MRSA transmission rate was

13.9 per 1000 patient days and during the intervention phase this was significantly reduced to 4.9 per 1000 patient days ($p<0.05$). The relative risk reduction was 0.65 (95% confidence intervals 0.28 – 1.07). Confounders were not collected and, because this was an observational study, despite the dramatic results, the contribution of the IDI-MRSA™ cannot be defined with certainty. However, because of the differences in the epidemiology of MRSA between the ICU and the general wards, rapid testing may be more effective in ICUs. The second ICU study with a positive impact of rapid testing on MRSA took place on the medical ICU (mICU) and the surgical ICU (sICU) of a primary and tertiary care hospital in Geneva, Switzerland (Harbarth S *et al*., 2006). The intervention was universal rapid MRSA screening using an in-house PCR assay (qMRSA) in combination with pre-emptive isolation. This study is discussed further in section 7.1.8. The third study was based in the USA and was described in section *7.1.1a(iii)*. This study had three consecutive intervention periods: a baseline period (I) without an MRSA admission screening programme followed by a second intervention period (II) when MRSA admission screening was introduced on the Intensive Care Units (ICUs) (ICU surveillance) and a final intervention period (III) when universal MRSA admission screening was introduced (universal surveillance) (Robiscek A *et al*., 2008). This study did not find a reduction in MRSA rates when rapid testing was introduced on the ICU compared to the baseline period when there was no MRSA screening. During all phases MRSA positive patients were placed under contact isolation. An explanation for the lack of a positive effect on the ICU is that MRSA decolonisation therapy was not applied to MRSA positive patients. This meant that the control of MRSA was reliant upon side room isolation, which is not always possible and was not measured in this study. The number of side rooms that were available was not given. There was also no data provided on compliance with infection control procedures such as hand hygiene. Finally, the turnaround time of the in-house PCR test during the ICU phase of the study was 2.5 days, which is much longer than the operational turn-around time for PCR tests reported by this thesis and others (Harbarth S *et al*., 2006; Cunningham R *et al*., 2007; Harbarth S *et al*., 2008; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Aldeyab MA *et al*., 2009; Hardy K *et al*., 2010). Indeed, this turnaround is comparable to or longer to that expected for conventional culture.

7.1.2b Surgical Wards

There are a number of differences in MRSA control between surgical and non-surgical patients. Firstly, elective surgical patients can be seen a few weeks before the planned procedure in order to prepare for it. This allows time for MRSA screening specimens to be taken and processed, and if the patient is colonised with MRSA, attempts are made to reduce the bio-burden prior to the procedure. Surgical patients are unique in two ways both of which can predispose to MRSA colonisation; they have a surgical wound and they often receive antibiotics peri-operatively. If the patient is colonised with MRSA, the peri-operative antibiotic prophylaxis can be modified to include an agent with activity against MRSA. However, once a patient is colonised, the presence of a break in the skin such as a wound usually makes MRSA decolonisation more difficult (Scanvic A *et al*., 2001).

Patients requiring emergency surgery obviously cannot be reviewed before admission. These patients may therefore benefit from an MRSA result obtained before the surgery starts produced by rapid admission screening. This would allow MRSA control measures to be instituted before the surgical incision is made. It may also allow decisions to be made about the patient's position on the operating list (usually last to avoid cross-transmission to other patients) or even to defer the procedure (if possible) until the patient has had some MRSA negative screening specimens.

Six of the ten wards included in this thesis housed surgical patients. However, the study was not powered for a sub-group analysis of these patients and from the data presented here it is not possible to specifically comment on the use of a rapid MRSA screening test on surgical patients. Six of the other published studies included surgical patients and some of these studies investigated only this type of patient. All surgical studies are limited by the fact that post-discharge cases of MRSA wound infection may be missed and a limitation of the study presented in this thesis is that there was no post-discharge wound surveillance, either actively by contacting or reviewing patients, or passively by searching for out-patient wound swab results.

A prospective interventional cohort study in Geneva investigated the effect of universal, rapid MRSA screening plus standard infection control precautions in comparison to standard infection control precautions without MRSA screening

(Harbarth S *et al*., 2008). A cross over design was used on elective and emergency surgical patients who were admitted for greater than 24 hours. The design was similar to the present study but omitted randomisation and discharge screening. Following a baseline period, half of the wards were assigned to the intervention and the remainder continued with standard precautions alone. After this there was a two-month wash out period and then the wards crossed over. The authors used their own molecular test, 'qMRSA' for the detection of MRSA. Patients were screened at the nose and perineum and other clinically indicated sites. If identified as MRSA positive they were given 5 days of decontamination therapy with nasal mupirocin and chlorhexidine body wash. There was no pre-emptive isolation for patients without a history of MRSA. The primary outcome measure was the number of nosocomial MRSA infections per 1000 patient days. The secondary outcome was the rate of MRSA surgical site infections per 100 procedures. There was prospective surveillance of surgical wounds by infection control nurses twice weekly and surgical site infection was attributed to the surgery if it occurred within 100 days of the procedure. A further secondary outcome was the nosocomial MRSA acquisition rate as detected on clinical cultures in a previously MRSA free patient. Adherence to admission screening was high (94%). The median turn-around time for the rapid result was 22.5 hours (interquartile range 12.2 – 28.2 hours). Admission screening identified 515 (5.1%) patients as MRSA positive, 26 (1.9%) of whom were identified at pre-admission clinic. The majority of MRSA positive patients (337, 65%) had not previously been identified as MRSA positive and would have been missed without systematic screening. Of 386 patients due to have surgery, 120 (31%) were identified after their surgery and so did not benefit from MRSA control measures prior to the procedure. Of the remaining 266, 115 (43%) had anti-MRSA peri-operative antibiotic prophylaxis. The rate of nosocomial MRSA infection was 1.11 per 1000 patient days in the rapid screening arm (93 patients) and 0.91 per 1000 patient days during the control arm (76 patients) (incidence rate ratio 1.2, 95% confidence interval 0.9 – 1.7, p=0.21). The authors adjusted for colonisation pressure, antibiotic selection pressure, use of alcohol based hand rubs, temporal trends and potential clustering effects and the result was virtually unchanged (incidence rate ratio 1.2, 95% confidence interval 0.9 – 1.7, p=0.29). The rate of surgical site infections was 1.14 per 100 procedures during the intervention phase and 0.99 per 100 procedures during the control phase (incidence rate ratio 1.2, 95% confidence interval 0.8 – 1.7) and the incidence of

nosocomial acquisition of MRSA was 1.69 per 1000 patient days during the intervention period and 1.59 per 1000 patient days during the control phase (incidence rate ratio 1.1, 95% confidence interval 0.8 – 1.4). It is notable that the majority (53, 57%) of the 93 patients, who developed any sort of nosocomial MRSA infection during the intervention phase had been MRSA negative on admission. Of the others, 23 (25%) had previously known MRSA carriage, and MRSA admission screening identified the remaining 17 (18%). Thus during the intervention period, 17 (5%) of 337 patients newly identified as MRSA positive on admission, 23 (13%) of 178 patients previously known to carry MRSA and 53 (0.5%) of 9678 patients found to be negative on admission developed MRSA infection during their hospital stay. None of the 26 patients identified as MRSA positive during their pre-operative outpatient visit developed an MRSA infection. All of them had received adequate decolonisation therapy and anti-MRSA peri-operative antibiotic prophylaxis. Again the majority, 41 (59%) of the 70 patients who developed an MRSA surgical site infection during the rapid screening period had no evidence of MRSA prior to the development of the infection. Of the remaining 29 patients who had all been identified as MRSA positive before their operation, 19 (66%, 27% of all rapid phase patients who developed a SSI) received anti-MRSA peri-operative prophylaxis and 12 (41%, 17% of all rapid phase SSI patients) received more than one day of decolonisation therapy. The paper does not state how many of these patients were previous positives and how many were picked up on admission screening. However, this data means that during the intervention phase, despite universal admission screening with a rapid test, 73% of the patients who developed an MRSA SSI did not get anti-MRSA prophylaxis and 83% did not get more than one day of decolonisation therapy. The same pattern of MRSA epidemiology emerged in the control arm; the majority (58, 76%) of the 76 patients who developed a nosocomial MRSA infection were not previously MRSA positive. Similarly, the majority (45, 75%) of the 60 patients who developed an MRSA SSI during the control arm had no evidence of MRSA prior to the development of the infection. Of the remaining 15 patients, who had all been identified as MRSA positive before their operation, 9 (15% but 60% of the 15) received anti-MRSA peri-operative prophylaxis and 14 (7% but 93% of the 15) received more than one day of decolonisation therapy. There are a few possible explanations for the development of MRSA infection in negative patients. The sensitivity of the qMRSA test has previously been reported as 96% (Francois P *et al*., 2007). However admission

screening may have failed to identify at least some of the patients who subsequently developed an MRSA infection either due to the sensitivity of the test or due to the less than 100% compliance with screening. In addition, despite the employment of a rapid test for admission screening, approximately one third of MRSA positive patients had their result after their surgery and one third who were known to be MRSA positive prior to theatre did not have anti-MRSA peri-operative antibiotic prophylaxis. The importance of the failure to introduce these MRSA control measures is highlighted by the fact that none of the patients who were identified in pre-admission clinic developed an MRSA infection and all of them had received anti-MRSA therapy before their operation. However, the pre-admission clinic patients will have received more than one day of decolonisation therapy and that may be the key factor. This is important because emergency surgical patients are unlikely to have time for a full course of decolonisation therapy prior to going to theatre. Further, the low infection rate in this cohort may be a reflection of the type of patients who are able to attend pre-admission clinic. These patients may be less prone to infection because they are having minor procedures and they are likely to be relatively well in comparison to emergency surgical patients. Thus the results from this group may not be applicable to emergency surgical patients. Finally, another possible, if not probable explanation for the overall result is the continued role for post-surgical cross transmission of MRSA and the subsequent development of infection. This means that in addition to systematic screening at the time of admission or pre-admission, rigorous application of infection control measures must continue throughout the patient's stay. Such actions, including good wound and line care should also reduce the proportion of patients who are previously or newly known to be MRSA positive who develop an MRSA infection (13% of the former and 5% of the latter in this study).

Another study of PCR screening for surgical patients in the UK found a significant reduction of *Staphylococcus aureus* bacteraemia and to a lesser extent MRSA wound infections relative to a historical control period during which there was no MRSA screening (Keshtgar MR *et al*., 2008). The intervention was over a one year period and the control data was from the six years prior to that. Elective and emergency surgical patients were screened at both anterior nares either in the pre-admission clinic or at the time of admission to hospital, respectively. Patients who were found to be MRSA positive, those with an unresolved PCR result due to have imminent surgery

and those without an MRSA screen result were started on decolonisation therapy of nasal mupirocin and chlorhexidine body wash. This therapy was expected to start 5 days before the surgery or the operation might be delayed. For those without a result, the therapy was continued until the result was known and for some it continued after the operation in order to complete the 5 days. MRSA positive results were telephoned to the patient or the ward and the peri-operative antibiotic prophylaxis was changed to teicoplanin and gentamicin. Patients with a temperature above 37.5ºC had a blood culture taken and the hospital wound surveillance team examined surgical wounds using a combination of observation, questioning of staff, examination of clinical notes and telephone or postal contact with patients. Surveillance continued for 1 – 2 months post-discharge. Eight hundred and fifty (4.5%) of the samples processed were MRSA positive. Patients having emergency surgery were more likely to be colonised (5.3%) than those having elective procedures (3.6%, p=0.001). The overall prevalence of MRSA carriage for surgical admissions was 4.0%. There were no changes in antibiotic prophylaxis during the study period nor were there significant trend effects for the prevalence of MRSA positivity on admission during the PCR screening period. The median turn-around time from the receipt of the specimen in the laboratory to the result was 21 hours (95% confidence intervals 21.0 – 22.5 hours). The median time from the start of decolonisation therapy to surgery was – 0.42 days (interquartile range –1.90 to 2.85 days) (a negative figure indicates that the surgery took place before the sample was processed). The overall rate of MRSA bacteraemia per 1000 patient days fell by 38.5% ($p<0.001$) compared with the control period. There was also a 30.4% reduction in MSSA bacteraemia ($p<0.001$), which was measured as a control. The rate of MRSA wound infection fell by 12.7% ($p<0.021$) compared with the control period. There was an insignificant fall in MSSA wound infections compared to 2005 ($p<0.430$) but there was an increase in MSSA infections by 12.7% ($p=0.006$) during the PCR screening period, which was confined to just one of the surgical specialities. There was no difference in the proportion of MRSA isolates resistant to mupirocin between the two periods. For 218 audited patients who were known to be MRSA colonised at or before surgery, 92 (42%) either received no topical decolonisation therapy or it was started after the surgery. MRSA was isolated from the surgical wound of 30 (33%) of these patients. The remaining 126 (58%) received at least one dose of decolonisation therapy before surgery and in 26 (20.6%) MRSA was later isolated from the wound ($p<0.05$). The authors propose that the

reduction in the MRSA bacteraemia figures is due to the shortened time to MRSA screening test result, which allowed decolonisation therapy and anti-MRSA antibiotic prophylaxis to be instituted quickly. They believe that the modest effect on wound infections may be attributed to the recurrence of MRSA after decolonisation therapy. This may also explain the lack of a sustained effect, reported by others, at 180 days post-procedure, which was initially seen on SSI rates at 30 days post-operatively (Robiscek). However that may also be because infection is more likely to occur sooner after the acquisition of MRSA than later. However there are flaws to this study. We know that with a median time of minus 0.42 days between the start of suppression therapy and surgery, most patients did not receive MRSA decolonisation therapy or modified antibiotic prophylaxis until after their procedure. This means that they did not fully benefit from the rapid result as planned; the impact upon intra-operative infections would be limited by the fact that screening was post-operative, though this strategy should prevent wound infections which occur as a result of inoculation of a discharging wound 24-48 hours after surgery. However there is an alternative explanation for the study's findings. In the four months immediately prior to the start of the PCR screening, patients had been moved to a new hospital building and this coincided with an increase in MRSA infections These figures had been included in the control data and they attempted to compensate for this by using data from the previous six years. The authors do not report how many MRSA wound infections occurred in MRSA negative patients nor the focus of the MRSA bacteraemia so further comment cannot be made on those factors. The fall in MSSA bacteraemia rates and the data presented for MSSA wound infections is noteworthy and supports the theory that potential confounders related to infection control, which were not measured, may be responsible for their overall findings.

Another interventional cohort study investigated the IDI-MRSA™ test in surgical patients and used a crossover design. This was set on seven wards of a large teaching hospital in the UK (Hardy K *et al*., 2010). Similar to the study in this thesis, the intervention was the use of the IDI-MRSA™ test (with simultaneous culture screening) compared to the control period when only culture testing was used (chromogenic agar). All patients admitted for greater than 24 hours were screened for MRSA at the anterior nares. PCR test results were released as soon as they were available and all MRSA positive results by any method were telephoned to the wards.

Patients who were MRSA negative on admission were screened every 4 days until they were discharged. MRSA positive patients were placed under contact precautions and given decontamination therapy with nasal mupirocin or naseptin, and triclosan body wash. There was no pre-emptive isolation in place. The primary outcome was the MRSA acquisition rate calculated as the ratio of patients admitted with MRSA to those who had acquired their MRSA during their hospital stay. Confounders including age, length of stay, proportion of patients undergoing emergency or elective surgery, source of admission, critical care admission and antibiotic usage were collected. The colonisation pressure was also accounted for. The overall colonisation pressure was 3.6%; 2.8% in the culture arm and 4.4% in the rapid arm. The turn-around time of the rapid test was 0.9 days and of the culture test was 3.3 days. 2.4% of patients during the culture phase and 1.9% of patients during the rapid phase acquired MRSA. After adjusting for potential confounders, the rapid method was found to have a significant effect on the number of patients acquiring MRSA during a ward stay with an estimated rate ratio of 1.49 (95% confidence interval 1.115 – 2.003, p=0.007) meaning that during the culture phase patients were 1.49 times more likely to acquire MRSA than during the rapid testing phase. The authors state that due to the shortened turn-around time, a greater proportion of patients in the rapid phase received decolonisation therapy (71.1%) compared to the culture phase (41.3%). They postulate that this was because during the culture phase most of the patients had been discharged by the time that their admission screen result was available. This was thought to be the reason for the fall in MRSA acquisitions. However the reported mean length of a patient stay during the culture phase was 7.2 days, which suggests that there was opportunity for some patients to receive this treatment. It may therefore be the case that compliance with this therapy was much higher during the intervention phase than during the control phase. This may mean that compliance with all infection control measures was higher during this period and that may be responsible for their findings. Further, all of the patients in this study were re-screened every 4 days and it is possible that this repeated screening contributed to the results either alone or in combination with the rapid screening. Universal, repeat screening is not normally performed in the NHS and is certainly one that would require additional resources. Finally six of the seven study wards saw a reduction of MRSA rates during the intervention phase. However, it is important to note that the transmission rate was 1.24 on the urology ward and 2 on the thoracic ward during the control phase. This is

consistent with a lack of MRSA control and a hyper-endemic/outbreak situation. The difference in transmission rates between the two arms was not statistically analysed for each individual ward. In fact it is possible that the individual wards did not find a significant difference between the control and intervention arms. It is certainly possible that the data from the urology and thoracic wards skewed the overall numerical result and that the conclusion that rapid screening significantly reduces MRSA rates in a controlled, endemic setting cannot be drawn.

An observational cohort study investigated IDI-MRSA™ screening in cardiothoracic surgery patients (Jog S *et al*., 2008). The control group was not screened for MRSA. During the intervention phase, there was no pre-admission clinic so elective patients were admitted to hospital the day before surgery. They were screened at the anterior nares and all patients were given pre-emptive decontamination with nasal mupirocin and triclosan pending the PCR result. Decontamination therapy was stopped if the PCR result was negative and patients received gentamicin and flucloxacillin peri-operative prophylaxis. MRSA carriers were given decontamination therapy for five days and teicoplanin and gentamicin peri-operative prophylaxis. The modified peri-operative prophylaxis was also given to patients with a previous history of MRSA, patients with an unknown MRSA status and those who had been on the ward for more than 96 hours since their last MRSA screen. MRSA positive patients were ideally isolated in a side room and their notes were tagged. MRSA infections were usually treated with vancomycin and rifampicin. Positive PCR specimens were reported as provisional positives pending the culture result and all positive and unresolved specimens were cultured. Patients found to be positive by the PCR method were then swabbed at the nose, groin and throat. There was prospective surveillance of surgical site infection (SSI) by a surveillance clerk. The confounders collected were the age and sex of the patient, the dates of admission and discharge, the duration of the operation, the surgeon, the underlying disease, the use of immunosuppressive drugs, the ASA score, the use of topical antimicrobials and the use of antibiotic prophylaxis. The outcome was the overall rate of SSI following cardiac surgery and SSIs due to individual organisms. Patients were followed up post discharge in outpatient clinics. The authors report an 89% compliance with screening in the study. The prevalence of MRSA colonisation on admission was 2.5% (19 patients). 18 of the 19 patients were screened before the surgery and 17 of them received topical decolonisation therapy

and anti-MRSA antibiotic prophylaxis. The overall rate of SSI fell from 3.30% to 2.22% with a significant reduction in the rate of MRSA infection from 1.15% to 0.26% ($p<0.05$, relative risk reduction 0.77, 95% confidence interval 0.056 – 0.95). None of the patients identified pre-operatively as MRSA positive developed an SSI. However two SSIs occurred after the introduction of screening, one was a deep sternal wound infection in a patient who had not been screened pre-operatively and hence did not receive topical decolonisation therapy or teicoplanin prophylaxis. Another occurred in a patient who had been PCR negative and was re-admitted from another hospital 20 days post-operatively. It is possible that this was a post-operative infection or that the patient was MRSA negative at the nose but was positive at another anatomical site. Two other patients who were MRSA negative developed wound infections at other sites and both may represent post-operative acquisition. There was no increase noted in the proportion of infections due to other organisms. The group concluded that PCR screening combined with suppression of MRSA at the time of cardiac surgery is associated with a significant reduction of subsequent MRSA SSI. However, the authors state that prior to the study, MRSA was responsible for >50% of their cardiothoracic SSIs. This suggests a problem with the control of MRSA on their cardiothoracic unit during the control phase and this may explain their statistical findings. It is possible that screening with culture would have had the same impact as PCR screening. However a rapid result was useful in this setting, as this hospital did not have pre-admission clinics. Finally, suppression therapy was given to all patients pending the PCR result. This may have been responsible for the findings alongside the issuing of modified peri-operative prophylaxis to patients without an admission or repeat screening result prior to theatre.

The final study solely on surgical patients was a retrospective chart review of otolaryngology patients (Richer SL & Wenig BL, 2009). The intervention was IDI-MRSA™ on a nasal swab. Colonised patients were given decolonisation therapy with nasal mupirocin and topical chlorhexidine for 5 days before the surgery. There was no change to the peri-operative antibiotic prophylaxis. During the control period there was no screening. An MRSA SSI was defined as an MRSA culture positive specimen from a surgical site within 30 days of the operation. In the phase before screening was introduced three (1.2%) of 241 patients developed post op infections, two (0.8%) of which were MRSA. During the screening phase 97 of 179 patients were screened

(54%) and 24 (24.7%) were colonised with Staphylococcus aureus. Two (8.3%) were identified as MRSA with an overall colonisation rate of 2%. No post-op MRSA SSI occurred after screening started. The retrospective chart review, the small number of patients studied, the low incidence of pre-intervention MRSA post-operative wound infections, the poor adherence to screening (46% of eligible patients were missed), and the lack of confounder data means that no clear conclusions can be drawn about the utility of MRSA PCR screening from this study despite the findings.

In summary, on surgical wards timely results from rapid admission screening are not always possible. In turn this means that patients do not necessarily benefit from decolonisation therapy and altered antimicrobial prophylaxis prior to their operation. Even if the result is received in time, there are conflicting results about the impact of these interventions on MRSA infection rates. This may be due to their suboptimal application prior to emergency surgery as there simply is not enough time to be effective. However, the benefits of such interventions have been quantified in studies of MRSA screening in pre-admission clinics. If the date of the pre-admission visit is sufficiently long enough prior to the planned date of surgery, culture screening is sufficient because a rapid result is not required. Where pre-admission clinics cannot be implemented, PCR testing may be of value. From the studies presented here, pre-admission screening is certainly associated with MRSA infection rates, though some of these findings may be a reflection of the wellness of patients who can attend such clinics. Whatever method of screening test is used, it cannot prevent post-surgical cross transmission of MRSA to previously negative patients or re-colonisation, both of which continue to be problematic. Neither can it deal with the development of infection in patients previously known to be MRSA positive. These can only be dealt with by good, basic infection control.

7.1.3 Anatomical sites screened for MRSA

There is debate amongst Medical Microbiologists about which anatomical sites should be screened for MRSA. The IDI-MRSA™ test is only licensed for use on swabs taken from the anterior nares (IDI-MRSA™ test product insert). It is notable that many authors have chosen to test specimens from multiple anatomical sites by PCR

(Harbarth S *et al*., 2006; Conterno LO *et al*., 2007; Harbarth S *et al*., 2008; Aldeyab MA *et al*., 2009), even though this may have been an off-license use. In the present study patients were screened at the nose, axillae and groins and other clinically indicated sites including wounds. All specimen types were processed by the PCR test. Patients were screened at these sites during both arms of the study. Authors tested at the same anatomical sites in both study arms in two of the five studies where patients were screened during the control phase (Harbarth S *et al*., 2006; Hardy K *et al*., 2010). In one study on a surgical ICU and a medical ICU, which concluded that rapid testing in combination with pre-emptive isolation reduces ICU acquired MRSA infections, patients were swabbed at the anterior nares and perineum, and if MRSA positive they were swabbed at other clinically indicated sites such as skin breaks during both phases (Harbarth S *et al*., 2006). Three studies screened at a greater number of sites during the culture phase than during the intervention phase (Conterno LO *et al*., 2007; Cunningham R *et al*., 2007; Aldeyab MA *et al*., 2009). One study on a UK ICU screened patients at the anterior nares during the PCR phase (using the IDI-MRSA™ test) and at the nose, throat, axillae, groin and wounds during the culture phase (Cunningham R *et al*., 2007). This study found a significant reduction of MRSA during the PCR phase. The second study found a non-significant reduction in MRSA rates during the PCR phase (Conterno LO *et al*., 2007). This discrepancy between the two arms may have affected the results. However this group performed PCR testing on specimens that had first undergone enrichment culture. In so doing they prolonged the turn-around time of the PCR test and that may have been responsible for the result (see section 7.1.4). They also removed control measures from patients who were PCR positive but culture negative and in so doing may have inadvertently allowed the ongoing transmission of MRSA from patients with a false negative culture result. Finally, one group processed throat swabs by culture only but otherwise, specimens were the same between the two arms (Aldeyab MA *et al*., 2009). Hospital acquired MRSA during the culture phase on the surgical ward was 22.1/1000 bed-days and during the PCR phase was 20.0 per 1000 bed-days ($p=0.69$). Hospital acquired MRSA during the culture phase on the medical/cardiology ward was 11.8/1000 bed-days and during the PCR phase was 20.3 per 1000 bed-days ($p=0.03$). This was a significant increase in MRSA detected during the PCR phase ($p<0.05$). It was due to an outbreak of MRSA as identified by pulse field gel electrophoresis and audits revealed a marked difference in compliance with infection

control procedures between the two phases. There is no reason to believe that the cardiology patients were more likely to be colonised at the throat and that therefore a large proportion of MRSA positive patients were missed during the PCR phase. Though this has been described in ICU patients, these patients will have been intubated and this is likely to increase the likelihood of colonisation (Batra R *et al*., 2008). The findings of all of these studies suggest that screening only at the anterior nares for PCR testing does not disadvantage the control of MRSA in comparison to multiple site testing by culture. It is possible that screening at the nose only by PCR is as sensitive at detecting MRSA positive patients as screening at multiple sites by culture.

7.1.4 Performance characteristics of the PCR tests

7.1.4a Turn-around time

The rapid test in the present study identified patients significantly faster thanconventional culture (21.8 hours versus 46.4 hours, $p<0.001$). This turn-around time was in keeping with other studies of PCR tests for MRSA screening (Harbarth S *et al.,* 2006; Conterno LO *et al*., 2007; Cunningham R *et al*., 2007; Harbarth S *et al*., 2008; Jog S *et al*., 2008; Keshtgar MRS *et al*., 2008; Robiscek A *et al*., 2008; Aldeyab MA *et al*., 2009; Richer SL & Wenig BL, 2009; Hardy K *et al*., 2010) and is an improvement on the time to result with standard culture. However healthcare is changing so that the patient's length of stay is as short as possible. It is interesting that many studies don't screen patients for MRSA unless their length of stay is >24 hours. With this in mind it is possible that a turn-around time of 22 hours is too long for MRSA control. As described in section *7.1.2b*, it proved too long for some patients requiring emergency surgery. Apart from the analysis of the time between an electronic result being available on the Smart Cycler and the same result being telephoned to the ward, in the present study we did not breakdown the turn-around time in to its other component parts, namely: 1) the time between the patient's admission to the ward and the screening specimens being taken, 2) the time between the specimens being taken and the specimens arriving in the laboratory, 3) the time between the specimens arriving in the laboratory and the processing of the specimens and 4) the time between processing the specimens and the results being available on

the Smart Cycler. Three studies did perform this analysis after the introduction of PCR screening (Harbarth S *et al*., 2006; Keshtgar MR *et al*., 2008; Aldeyab MA *et al*., 2009). The time between ward admission and screening was variable. In one study it was a median of 13.4 hours (interquartile range (IQR) 4.8–21.6) in the culture phase but 6.3 hours (IQR 0.6–10.3) in the PCR phase, which was statistically significantly different ($p < 0.001$) (Harbarth S *et al*., 2006). In another study the same time measurement was reported as 0.24-0.25 hours (depending upon the ward) in the culture phase and as short as 0.05-0.67 hours in the PCR phase (no statistically significant difference) (Aldeyab MA *et al*., 2009). Another statistically significant difference was in the time from receipt of the screening specimen in the laboratory to result notification which was 71.8 hours (IQR 47.9–94.6) in the culture arm and 7.2 hours (IQR 6.3–22.2) in the PCR phase ($p<0.001$) (Harbarth S *et al*., 2006), and in another study from admission to the result being telephoned which took a median of 19.3 hours (IQR 13.8-23) on the surgical ward during the PCR phase but 51.8 hours (IQR 44.4-69) during the culture phase ($p<0.001$), and 22.7 hours (IQR 19.8-23.8) on the medical ward during the PCR phase but 42.2 (IQR 40.3-69.9) during the culture phase ($p<0.001$) (Aldeyab M et al., 2009). The time from screening to arrival in the laboratory did not differ between the culture and PCR arms and also varied between studies with a median time of 3.2 - 3.6 hours in one study (Harbarth S *et al*., 2008) and 13.7 hours in another study (Keshtgar MR *et al*., 2008).

Given that PCR screening tests are often referred to as 'rapid' tests, it is to be expected that the specimen processing is shorter in the PCR phase. However the degree of difference between PCR and culture processing depends upon the culture method used, which varies between UK hospitals, as well as the PCR method. Some chromogenic agars can produce a negative result after 24 hours of incubation. With high negative predictive values (92%-95% if MRSA prevalence is high) management decisions for MRSA negative cases can be made at this point (Malhotra-Kumar S *et al*., 2010). Some manufacturers suggest that as sensitivity is good (93.7%) after only 18 hours of incubation, management decisions for MRSA positive cases can be made at that time also (Brilliance MRSA2 'Spend even less time confirming false-positives' accessed at http://www.oxoidhai.com/mrsa/brilliance-mrsa-2-resource-centre). This suggestion does not factor in confirmatory tests. However such media usually require 48 hours of incubation which increases the sensitivity but decreases the specificity with negative impact on both time to result and cost-efficiency. Those tests that use an

enrichment broth step with sub-culture on to agar, as in the present study add 18-24 hours to this process. However, to reduce processing time it is possible for clinical staff to swab the patient, agitate the swabs in the enrichment broth at the point of care, discard the swabs and send the broth to the diagnostic laboratory (Rao GG *et al.*, 2007). A direct tube coagulase on the broth also reduces processing time.

There is no obvious reason why the time between admission and screening should differ between the two arms as reported by Harbarth *et al* and Aldeyab *et al*. Perhaps the ward staff in those studies were motivated by the promise of a rapid result, less cross-transmission of MRSA and the chance to optimise side room use. Perhaps the investigators influenced the result by their presence on the wards. It may be the case that the time between admission and screening was shorter in the intervention arm in the present study, which may be a consequence of investigator influence. However, since the turn-around time in the present thesis concurs with those in ten other studies, the probability is that this is an unbiased result. A portion of the turn-around time is due to the transport of the specimens to the laboratory (Harbarth S *et al*., 2006) therefore one way to reduce it is to develop point of care testing. The first test of this kind is on the market in the form of the Cepheid GeneXpert system (Cepheid, Sunnyvale, CA, U.S.A). This is a random access system thus avoiding the need for batching and purports to produce a result in 70 minutes at the point of care. However it is currently more expensive than available methods at a commercial rate of US$42 per test and instrument costs of US$ 25,000 – 35, 000 (Cepheid Xpert MRSA product literature).

Two studies had a notably long turn-around time. The first was in a study in the USA (Robiscek A *et al*., 2008). An in-house PCR method used during one phase had a turn-around time of 2.5 days. The reason for this is not given. When this test was introduced on to the ICUs, there was no difference in MRSA rates in comparison to the control phase (see section *7.1.1a(iii)*). In the second study, the PCR test was performed on specimens that underwent overnight enrichment culture first (Conterno LO *et al*., 2007). This study was conducted on three campuses in Ottawa and the primary outcome measure was the monthly incidence of nosocomial MRSA colonisation or infection per 100,000 patient days. Nosocomial acquisition was defined as any patient in whom MRSA was detected from a screening swab specimen

or clinical specimen obtained 48 hours or more after admission. However if the patient had been in the hospital within the last two months and had a positive admission screen then they were considered to have acquired their MRSA in the hospital as long as they had not come from another healthcare facility within those two months. Patients at high risk of MRSA carriage and the contacts of patients identified as MRSA positive were screened at the anterior nares, rectum, open skin lesions and catheter exit sites. There was no pre-emptive isolation. During the rapid phase, the PCR result had to be confirmed with culture in order for the contact precautions to continue. The swabs were pooled in a selective broth that was incubated overnight and then either sub cultured on to sheep blood agar in the control phase or during the intervention phase 50μL of the broth was processed using the IDI-MRSA™ test. This group validated the use of the IDI-MRSA™ on broth in this way before the study and the test performed well. The nosocomial MRSA transmission rate was calculated as the ratio of patients with nosocomial acquisition of MRSA (colonisation or infection) to the number admitted with colonisation or infection. Overall the rate of nosocomial MRSA colonisation or infection was 0.37 per 1000 patient days (range 0 – 1.3). The mean time from screening to the initiation of contact precautions decreased significantly from 3.8 days to 1.6 days ($p<0.001$) during the intervention phase. However, there was an insignificant reduction of nosocomial MRSA transmission during the rapid screening phase of 0.14 cases per 1000 patient days per month (95% confidence intervals –0.18 to 0.46, $p=0.39$). The smaller than expected reduction in MRSA may have been due to the amount of time taken to institute the MRSA control measures because even though this was faster than during the control period, at approximately one and a half days it still may have been too long. It is also possible that because this group removed control measures from patients who were PCR positive but culture negative, that they removed control measures from some truly MRSA positive patients and thus allowed the on-going transmission of MRSA. Finally, this group did not measure any confounders but they surmise that new technology is not the solution to MRSA in the absence of good infection control.

7.1.4b Unresolved rate

Only 0.09% of the tests did not produce a result due to inhibition of the PCR reaction that could not be resolved. This is much lower than the 1% rate reported by the manufacturer and the 0.9% rate we found in our validation study for the use of the IDI-MRSA™ test on pooled and non-nasal specimens (Jeyaratnam D *et al*., 2008). The only change to the IDI-MRSA™ methodology between the validation study (see Study 1) and the clinical trial was that a duplicate set of swabs was sent for processing in the trial. In the validation study all of the specimens had already been processed by culture. It is possible that inhibitors of the PCR reaction increased during storage or as a result of processing by culture, prior to processing by PCR in the validation study. However, this does not explain the difference between the results presented here and the data reported by the manufacturer. The reason for the low unresolved rate presented here is undetermined.

7.1.4b Sensitivity, Specificity, NPV and PPV

The IDI-MRSA™ performed well as an MRSA screening test in the present trial. The sensitivity of the IDI-MRSA™ test in comparison to conventional culture was 87.8%, the specificity was 96.3%, the negative predictive value (NPV) was 99.4% and the positive predictive value (PPV) was 55.1% in a setting where the admission prevalence was 6.7%. The specificity and NPV were high (96.3% and 99.4% respectively) and similar to results reported by other clinical studies (Cunningham R *et al*., 2007; Conterno LO *et al*., 2007; Aldeyab *et al*., 2009; Wassenberg MWM *et al*., 2010; Hombach M *et al*., 2010). At these prevalence levels, the IDI-MRSA™ should reliably identify MRSA negative patients.

The sensitivity of 87.8% is slightly lower than that found in other validation studies with similar specimen types (De San N *et al*., 2007; Reyes R *et al*., 2006; Warren DK *et al*., 2004; Zhang *et al*., 2007). It is also lower than sensitivity results reported by other clinical studies (Cunningham R *et al*., 2007; Conterno LO *et al*., 2007; Hombach M *et al*., 2010). Two of those studies tested specimens from non-nasal sites (Conterno LO *et al*., 2007; Hombach M *et al*., 2010). One tested aliquots of enrichment broth in which screening swabs had first been inoculated, which may explain the PCR sensitivity result of 96% (Conterno LO *et al*., 2007). Another study only swabbed the nares but does not give details of the specimen processing, which

may suggest that they followed the manufacturers' instructions. They reported a sensitivity of 100% (Cunningham R *et al*., 2007). The third study specifies that swabs were transported in liquid Stuart's medium and a variety of specimens were tested (Hombach M *et al*., 2010). The sensitivity of the PCR test improved to 100% from 83.4% during a validation of their method. They state that the improvement may have been due to the use of liquid Stuart's medium to transport the swabs to the laboratory rather than Amies' medium, which was used in their validation study (Hombach M *et al*., 2010). The transport of swabs in Amies' medium in the studies presented in this thesis may have reduced the sensitivity of the test since solid media may reduce the elution of staphylococci (Hombach M *et al.,* 2010).

Though most groups reported the positive PCR results as provisional whilst awaiting the result from the matched culture specimen, they did not specifically report problems with the PPV. However, the poor PPV for MRSA PCR screening tests is not unique to this study (Warren DK *et al*., 2004; Bishop E *et al*., 2006; Harbarth S *et al*., 2006; de San N *et al*., 2007; Herdman MT *et al*., 2009). Conterno LO *et al* validated the IDI-MRSA™ for use on specimens that were incubated overnight in enrichment broth (Conterno LO *et al*., 2007). In their pre-study analysis, the PPV for this method was 90%, however in practice this fell to 65% and from thereon they confirmed every PCR positive result with culture. This led to increased costs because of contact precautions for patients considered to have a false positive results (50% of the increased costs) and culture confirmation. However, predictive values are a function of the local prevalence, so this will be less of an issue for areas with a higher prevalence of MRSA and consequent higher PPV values. It has been suggested that in a setting of a low prevalence of MRSA carriage on admission, a negative PCR result is a true predictor of MRSA status and future risk but a positive PCR result should be confirmed by culture (Herdman MT *et al*., 2009). This should be done before infection control measures are decided on that carry a risk to the patient such as cohort nursing or delaying essential procedures. Therefore, with these performance characteristics, a rapid negative result allows some resource savings by releasing MRSA negative patients from pre-emptive isolation but due to the low PPV it may not impact significantly on true MRSA positive carriers.

Molecular methods can give false-positive results if non-specific sequences are amplified. Rupp J *et al*. (2006) pointed out that single locus PCR methods for MRSA, such as the IDI-MRSA™ is potentially especially liable to this problem and described an MSSA isolate containing only small fragments of the right extremity of SCC*mec* that resulted in false-positive reactions with a similar test. These *Staphyloccus aureus* strains have lost the *mecA* gene from the cassette and thus resistance to meticillin. Thus phenotypically and biologically the isolate is meticillin sensitive. The PCR tests are not designed to detect whether or not the cassette still holds the *mecA* component or not. Primer sets target the right hand portion of the chromosomal cassette (to identify methicillin resistance) and the *OrfX* (to identify *S. aureus*). The test therefore gives a positive result in these phenotypically MSSA strains. Approximately 25% of 158 culture negative/IDI-MRSA™ positive specimens identified in this study cultured MSSA on further investigation. It is possible that some or all of these MSSA isolates contained the right extremity of *SCCmec* and that these were false positive IDI-MRSA™ tests. Desjardins *et al*. (2006) used the IDI-MRSA test to examine nasal and rectal swabs pooled in a selective broth. Out of 298 IDI-MRSA assay-positive broths, 103 could not be confirmed by culture; MSSA was recovered from 77 of these 103 and gave positive results with IDI-MRSA. There were 17 different PFGE genotypes amongst these MSSA and about half of them were similar to common local MRSA genotypes, including the CA-MRSA clones USA 100 & 500, which may have been MSSA strains that had not yet acquired *mecA.* It is notable that in a prospective study in the same institution as the study presented in this thesis, culture negative/IDI-MRSA™ PCR positive tests were significantly less likely to occur in patients with a history of MRSA and more likely to occur in patients with a history of MSSA (Herdman MT *et al*., 2009). Another explanation for the culture negative/IDI-MRSA™ positive result could be meticillin resistant coagulase negative staphylococci causing a false positive result. This has been demonstrated experimentally using the IDI-MRSA™ and GeneXpert assays (Malhotra-Kumar *et al*., 2010). These authors report that preliminary sequencing of the *orfX*-SCC*mec* junction in meticillin resistant coagulase negative staphylococci that caused false positive PCR results has shown high homology to MRSA. Thus there is cross-reactivity of single-locus PCR assays with some MSSA but also with some methicillin resistant coagulase negative staphylococci, which can affect the performance of these assays (Malhotra-Kumar S *et al*., 2010).

In the study by Herdman *et al*. and the present one it is also feasible that some of the poor PPV was due to a false negative result with the MRSA selective broth. MRSA was subsequently cultured from 20% of the culture negative/IDI-MRSA™ positive tests in the present study. Herdman *et al*., studied pooled IDI-MRSA™ swabs using the same method described in the present thesis and found that culture negative/PCR positive specimens had a statistically significantly higher threshold of PCR signal intensity (ct number) than in PCR positive/culture positive patients suggesting that a lesser amount of DNA was present in the former group of tests (Herdman MT *et al*., 2009). This suggests that the PCR test is able to detect MRSA at levels too low for reliable detection by broth enrichment. The low positive predictive value has been contested by investigators who used MRSA results from other specimens taken from other anatomical sites to resolve discrepant results (Hombach M *et al*., 2010, Smith MH *et al*., 2010). By doing this the PPV of the BD GeneOhm™ test has been calculated to increase from 82.4%-87.5% to 93.3-94.1%. Consequently PCR detection was defined by those authors as the gold standard presumed to be due to better detection limits. In addition, swabs from wounds, axillae, the vagina and the throat were found to be more likely to produce a result discrepant with the paired culture result (Hombach M *et al*., 2010). It was hypothesised that this may be due to lower MRSA colonization rates and, therefore, lower pre-test probability for these sites compared with the nares. In another study culture negative/PCR positive tests were not significantly associated with the use of decolonisation therapy, thus opposing the hypothesis that the PCR assays may be detecting dead MRSA DNA (Herdman MT *et al*., 2009). We know that some of the culture negative/PCR positive tests in the present study occurred because the broth contained ciprofloxacin and could therefore not isolate MRSA strains that are ciprofloxacin susceptible. The majority (68.8%) of the MRSA isolates yielded from culture negative/IDI-MRSA™ screens after further testing were ciprofloxacin susceptible. The ciprofloxacin susceptibility will not have affected the ability of the IDI-MRSA™ assay to identify an isolate as MRSA.

A molecular assay, which targets the mecA gene, will miss an MRSA isolate that is a Beta lactamase hyper-producer.,Though these are rare, they may be responsible for some of the discrepant results described here. In addition, because single locus systems target SSC*mec* elements, they may give false-negative results if – as is increasingly the case – local strains of MRSA appear of unusual or variant SSC*mec*

types (Francois P *et al*, 2007, Rossney AS *et al*, 2007a). Francois *et al.* (2007) found the IDI system to have poor specificity when tested against 93 MRSA and 89 MSSA of diverse genetic backgrounds. Their own multi-locus qPCR system was not affected by this problem, and they warn that users of *orfX*-SSC*mec* based assays should repeatedly monitor local epidemiology to minimize the risks of failing to detect emerging MRSA clones.

In conclusion, the origin of discrepant PCR and culture results is complex. Culture positive/IDI-MRSA™ negative results were not investigated further. There is no single explanation for the culture negative/IDI-MRSA™ positive tests in the present study. Given the results of the subsequent investigation of these isolates, it seems likely that false positive PCR results due to MSSA and meticillin resistant coagulase negative staphylococci as well as false negative culture results due to ciprofloxacin susceptible strains of MRSA and the lower limits of detection of broth culture are all involved. Without further investigation it is not possible to draw firm conclusions but it is likely that the true PPV of the IDI-MRSA™ assay is not as low as the 55.1% calculated in this trial.

7.1.5 Use of culture detection

Five studies did not have any MRSA screening during the control phase (Harbarth S *et al*., 2008; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008; Richer SL & Wenig BL, 2009). For all of these studies, bias is introduced by the fact that only patients in the test group were screened and this may result in a greater compliance with infection control procedures in this group or a greater likelihood to test for MRSA infection in these patients. Only one of these studies (Harbarth S *et al*., 2008) measured confounders in order to compensate for this. Four found that PCR screening reduced MRSA rates but it is not clear if screening with culture would have had the same impact. The fifth study was a controlled trial, which found no impact on MRSA rates with PCR screening. Those that did use culture in the control arm differed in the method chosen: oxacillin resistant screening agar, colistin-salt broth and VITEK 2 (Harbarth S *et al*., 2006), sheep blood agar and selective broth (Conterno LO *et al*., 2007), sodium chloride broth and mannitol salt agar

(Cunningham R *et al*., 2007), and chromogenic agar (Aldeyab MA *et al*., 2009; Hardy K *et al*., 2010). None of these studies used a selective medium containing ciprofloxacin, such as the broth used in the present study, which may have missed community acquired MRSA strains. Like the study in this thesis, all of them used methods, which are widely used in hospital diagnostic laboratories.

7.1.6 Prevalence of MRSA

Most of the other published studies have an admission prevalence rate similar to that described in the present study: 6.7% (Harbarth S *et al*., 2006), 7% (Cunningham R *et al*., 2007), 5.1% (Harbarth S *et al*., 2008), 8.3% (Phase II) and 6.3% (Phase III) (Robiscek *et al*., 2008), and 6.8% control and 7.3% intervention (Aldeyab MA *et al*., 2009). Those that had a different prevalence rate were usually studies confined to surgical patients, and the rates were lower (Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Hardy K *et al*., 2010). Since these studies were undertaken the prevalence rate of MRSA carriage determined by universal screening has fallen to 1-2% (Collins J *et al*., 2011). This may be for two reasons: firstly, because untargeted universal screening results in a larger denominator with most patients low risk for MRSA carriage, rates will be lower. Secondly the MRSA control programme in England has been successful: fewer patients are acquiring MRSA in hospital, fewer carriers are being discharge and thus the cycle of revolving door re-admission of carriers has also fallen. Low prevalence rates will affect the performance of characteristics of all screening tests and the cost-efficiency of screening programmes. One paper has presented trend data (Cunningham R *et al*., 2007) but the rest including the one presented here have not. Acute outbreaks of MRSA were reported during the trial presented in this thesis, which suggests that the IDI-MRSA™ test cannot necessarily prevent or arrest such events. However, others have reported problems with MRSA control before the introduction of rapid testing (Jog S *et al*., 2008; Keshtgar MR *et al*., 2008). Another group reported transmission rates of greater than one during the control phase of their study (Hardy K *et al*., 2010). All of these groups found a reduction in MRSA rates during the PCR phase. However their findings could be as a consequence of the statistical phenomenon of regression to the mean. Similarly, a clinical area with overall low rates of MRSA such as a surgical ward is subject to the

effects of chance variability, regression to the mean, and low power to detect genuine underlying changes (Spiegelhalter DJ, 2005). Harbarth *et al*. concede this point in their publication because they had low MRSA infection rates (Harbarth S *et al*., 2008).

7.1.7 Use of MRSA decolonisation therapy and antibiotic prophylaxis

The majority of studies, including the one presented here applied MRSA decolonisation therapy to MRSA positive patients (Harbarth S *et al*., 2006; Cunningham R *et al*., 2007; Harbarth S *et al*., 2008; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008; Richer SL & Wenig BL, 2009; Hardy K *et al*., 2010). In most of the studies decolonisation therapy was given in the intervention and control arms. However in some of the studies this therapy was only given in the intervention arm because patients were not screened systematically in the control arm (Harbarth S *et al*., 2008; Jog S *et al*., 2008; Keshtgar MR *et al*., 2008; Robiscek A *et al*., 2008; Richer SL & Wenig BL, 2009). This makes the impact of a rapid result by means of PCR testing difficult to interpret. In one study, all of the patients in the intervention arm were given MRSA decolonisation therapy prior to and pending the MRSA PCR result (Jog S *et al*., 2008). This was stopped if the patient was MRSA negative. During the control arm there was no screening and thus MRSA decolonisation therapy was not systematically and universally applied. The authors concluded that PCR screening reduced the incidence of MRSA. However the role of the universal application of MRSA decolonisation therapy at least until the PCR result was back is not clear and it is possible that this is the intervention that caused MRSA rates to decrease during the intervention arm. It should also be noted that the universal application of decolonisation therapy is undesirable due to the risk of the emergence of resistant strains. In another study, where decolonisation therapy was only given to patients in the intervention arm, it was given to those who were found to be MRSA positive by PCR but also to those with an unresolved PCR result due to have imminent surgery and those without an MRSA screen result (Keshtgar MR *et al*., 2008). This therapy was expected to start 5 days before the surgery or the operation might be delayed. For those without a result, the therapy was continued until the

result was known. Applying decolonisation therapy to those who did not have a confirmed MRSA positive result would also have been a departure from protocol in the control arm and in routine practice, and may have influenced the result. The authors have not published what proportion of their patients fell in to this category. However, they did not apply decolonisation therapy universally as in the study by Jog *et al*. This group found that the rate of MRSA bacteraemia and wound infections and MSSA bacteraemia (a control) fell significantly during the PCR phase. MRSA decolonisation therapy will have reduced the nasal carriage of MSSA and may be responsible for the fall in bloodstream infections. Some of the studies reported on rates of Mupirocin resistance (Keshtgar MR *et al*., 2008) or rates of successful decolonisation (Cunningham R *et al*., 2007) between the two study arms and neither of these were reported to be responsible for the study findings. The study presented in this thesis did not report on these rates.

None of the published studies used systemic antibiotics to reduce the burden of MRSA in colonised patients however some of the studies included patients on surgical wards and as a result these patients received peri-operative antibiotic prophylaxis. This was usually modified if the patient was MRSA positive prior to the surgery. In one study (Jog S *et al*., 2008) the modified peri-operative prophylaxis was also given to patients who had been on the ward for more than 96 hours since their last MRSA screen. As patients in the control arm in this study were not screened, this means that many more patients in the intervention arm received an alteration to these antibiotics than in the control arm. This is another confounder that may have contributed to the positive findings.

7.1.8 Pre-emptive isolation

One study took place in the medical ICU (mICU) and the surgical ICU (sICU) of a primary and tertiary care hospital in Geneva, Switzerland (Harbarth S *et al*., 2006). This is the only study other than the study presented in this thesis to clearly and systematically employ pre-emptive isolation. The ICUs were reported to have endemic levels of MRSA. The intervention was universal rapid MRSA screening using an in-house PCR assay (qMRSA) in combination with pre-emptive isolation.

Patients who stayed on the ICU for greater than 24 hours were included in the study. On both of the ICUs during the historical control period, only patients at high risk for MRSA carriage were screened for MRSA. The method of detection was conventional culture using Oxacillin resistant screening agar, colistin-salt broth and VITEK 2. On the surgical ICU high-risk patients were pre-emptively isolated during the control phase. During the one intervention period universal admission screening, universal discharge screening and universal pre-emptive isolation were introduced. The mICU had two sequential intervention periods; during the first, all patients were screened for MRSA carriage on admission and discharge, and during the second intervention period the additional step of pre-emptively isolating all patients was introduced. During both phases, patients were swabbed at the anterior nares and perineum, and if MRSA positive they were swabbed at other clinically indicated sites such as skin breaks. The primary outcome measure was ICU acquired MRSA infections, which were assessed by a dedicated study nurse. The overall prevalence of MRSA carriage on admission was 6.7% and the universal screening identified 55 patients as MRSA positive who would have previously remained unidentified unless a clinical specimen yielded MRSA. After adjusting for colonisation pressure, the universal admission screening and pre-emptive isolation policy was associated with a reduction in mICU acquired infections (relative risk 0.3, 95% confidence interval 0.1-0.7). However, it is notable that that the rate of mICU acquired infection dropped significantly only after the introduction of pre-emptive isolation and not with the introduction of qMRSA alone. However, on the sICU, where the policy during the control period had been to isolate high risk patients there was no significant effect on the infection rate during the intervention period when PCR screening was introduced (relative risk 1.0, 95% confidence interval 0.6-1.7). The effect of pre-emptive isolation is dramatic and clear from this study; the rate of acquired infection was reduced by pre-emptive isolation only. PCR screening did not reduce these rates and did not add to the effect of pre-emptive isolation. The pre-emptive isolation of patients with a high risk of MRSA carriage appears to be critical to the control of MRSA (Bootsma MCJ *et al*., 2006) and is a component of the successful Dutch 'Search and Destroy' policy (Wassenberg MWM *et al*., 2010). However there has been no investigation in a clinical setting of the relative contribution of each component of this MRSA control programme. Only the study presented here and the study by Harbarth *et al* (Harbarth S *et al*., 2006) have assessed the effect of PCR testing on MRSA acquisition where high-risk patients were

pre-emptively isolated. A second study by Harbarth *et al*. stated that 'no pre-emptive isolation was instituted for patients without a history of MRSA carriage' (Harbarth S *et al*., 2008) suggesting that a more limited group of patients were included in that policy compared to those in their first study and the study in this thesis. The second study by Harbarth *et al*., did not show a reduction of nosocomial MRSA infections after the introduction of PCR screening (Harbarth S *et al*., 2008). In the present study, MRSA transmission rates were in keeping with good infection control rates and there was no reduction of MRSA acquisition rates when PCR testing was introduced. It is probable that the pre-existing policy of pre-emptive isolation of patients at high-risk for MRSA carriage heavily influenced the findings in the present study. Approximately one third of patients subsequently identified as MRSA positive by culture had been pre-emptively isolated. It can be argued that pre-emptive isolation is more rapid than the IDI-MRSA™ test and thus negates the need for such a test with respect to the control of MRSA rates. The data from the present study lends itself to that theory. However both studies demonstrate that the use of rapid screening realises the potential of side rooms by liberating them once a patient who is pre-emptively isolated is rapidly identified as MRSA negative (see section 7.1.9). It must be noted that the study by Harbarth *et al*. used a historical control period and did not collect data on potential confounders. However it supports the findings of the randomised trial presented here which similarly did not find a significant reduction of MRSA rates with rapid MRSA screening in a setting with an identical colonisation pressure, a very similar turn-around time for the rapid test and which employed pre-emptive isolation.

Another English prospective, cross-over study found a statistically significant reduction of MRSA acquisition when IDI-MRSA™ testing was introduced (Hardy K *et al*., 2010). This study has been discussed in detail in section *7.1.2b*. The wards in that study had between 20 and 34 beds, arranged in bays of six beds with two to five single isolation rooms, and 17% of the MRSA positive patients were isolated in one of these rooms. Data concerning cohort nursing on the wards was not provided. A similar total number of MRSA positive patients were reported in that study (721) compared to the present study (804). The authors state that in most UK hospitals there are a small number of single rooms available for patient isolation and this is why the majority of the MRSA positive cases in their study were not isolated. They suggest that the reduction of MRSA found in their study was due to the rapid identification of

carriers by the PCR test. However the rates of MRSA on some of the wards in that study were high and transmission rates in some areas were greater than or close to 1. This suggests sub-optimal infection control and data on rates of infectious diarrhoea or other organisms requiring isolation, which may also have been at higher levels, have not been given. Such cases will have placed pressure upon side rooms. Isolation of patients with diarrhoea is usually prioritised over MRSA in infection control policies due to the high infectivity. Further, now that the prevalence rates of MRSA have fallen (Collins J *et al*., 2011), which will in-part be due to improved infection control, the number of side rooms required for MRSA positive patients will also decrease. As a result, a policy of pre-emptive isolation of patients at high risk for MRSA should be reconsidered by NHS hospitals.

7.1.9 Resource Use: Isolation Days

In the present study there was a significant difference in the number of inappropriate isolation days between the control and intervention arms (399 versus 277, respectively $p<0.001$) attributed to the rapid identification of patients as MRSA negative during the intervention phase. Approximately three-quarters in each arm were spent in side rooms and the proportion of patients pre-emptively isolated was similar between the two arms. At a prevalence rate of 6.7%, the NPV of 99.4% of the IDI-MRSA test was sufficiently high that it would have allowed MRSA negative patients to be removed from pre-emptive isolation once the result was available. Over 10 months this would have released 122 side-room days for use by other patients. Another study, which pre-emptively isolated patients, investigated their own rapid PCR method, 'qMRSA' (Harbarth S *et al*., 2006) (see section 7.1.8). This group calculated that on the surgical ICU qMRSA saved 1227 unnecessary (in the present study called 'inappropriate') pre-emptive isolation days for 245 MRSA negative surgical patients.

The number of inappropriate open days between the two arms was not significantly different between the two arms (389 in the control arm versus 351, $p=0.08$). Patients were treated as MRSA positive even if the screening results between the two detection methods was discordant. One hundred and fifty nine patients had an MRSA screening

result that was culture negative but PCR positive, which may have been a false positive PCR result. When the number of inappropriate open days was calculated using only culture positive results (potentially 'true positive' results) the difference between the two arms was statistically significantly different (389 in the control arm versus 213 in the rapid test arm, $p<0.001$). For 'true' MRSA positive patients (i.e. where the IDI-MRSA™ concurred with the culture result), the number of days where MRSA positive patients were being nursed on the open ward without any control measures prior to their admission screening result was reduced by 176 days over 10 months. These patients had MRSA control measures including isolation applied sooner than their counterparts in the control arm. The time during which susceptible patients were exposed to these MRSA carriers was thus reduced by 176 days over the 10 study months. This was the original goal of rapid testing though the present study did not observe a reduction of MRSA acquisition rates. However, the patients identified by IDI-MRSA™ testing only, spent 138 inappropriate isolation days on the ward. This group, which is unique to the intervention arm, are a pressure on resources as they require a side room for isolation, decolonisation therapy and the donning of gowns and gloves by those looking after them. The PPV of the IDI-MRSA™ test was 55.1% when compared to culture. Some of these patients are therefore false positive cases and will unnecessarily occupy a side room for MRSA control. Thus the beneficial effect on side rooms seen in the rapid test phase due to the reduction of the number of inappropriate isolation days is reduced if rapid tests falsely identify patients as MRSA positive. What is more, some patients do not like to be placed in a side room because they feel socially isolated, and the quality of patient care may be reduced (Kirkland KB and Weinstein JM, 1999).

A three-phase study that took place in the USA also looked at isolation room use (Robiscek A *et al*., 2008). They found a reduction in hospital associated MRSA disease per 1000 patient-days during the third phase when universal, PCR screening throughout their hospital was introduced. They reason that the beneficial effect of universal surveillance is due to the contact isolation of MRSA positive patients in side rooms, which in their study numbered 11,545 patient-days. However, this was only directly observed for the first two-thirds of the universal surveillance period and for much of the study the authors presumed that isolation had occurred immediately after a positive result was reported, which may not have been the case. However, such direct observation can introduce bias and it may make the isolation of patients more

likely during the observed period. Using the turn-around time of the test, the authors estimated the number of contact isolation days for MRSA positive patients that would have occurred during the baseline period (when clinical culture specimens only identified MRSA positive patients) as 2036 contact isolation days. The difference in the number of isolation days between the two phases (11,545 – 2036 = 9418) was used to calculate the expected reduction in MRSA transmission during the two phases. During the baseline period, without contact precautions an estimated 0.14 transmissions would be expected to occur per un-isolated patient day (Jernigan JA *et al.*, 1996). Therefore 9418*0.14 = 1319 which is the expected number of transmissions. During the universal screening phase 0.00875 transmissions would be expected to occur per isolated patient day giving 9418*0.00875 = 82 expected transmission. The reduction between the baseline and the period when universal screening, decontamination therapy and active education programmes were introduced was –5.0 per 10 000 patient days (95% confidence interval -6.6 to -3.5). The authors state that the magnitude of difference in transmissions from 1319 to 82 due to contact precautions alone fits with the reduction in MRSA that they observed in their study. This study clearly illustrates the impact that universal screening can have on side room use.

The present study and that by Harbarth *et al*. (Harbarth S *et al*., 2006) have highlighted the impact of rapid admission screening on a policy of pre-emptive isolation. It appears that rapid testing optimises side room use when pre-emptive isolation is in practice, provided that the specificity of the test is good. Pre-emptive isolation can be implemented immediately after a patient arrives in hospital on the proviso that the patient's MRSA risk factors are assessed. These can be done as part of the triage or clerking in process on arrival. The pre-emptive isolation would ideally be in a side room and at GSTT there are a reasonable number; in the present study side rooms were available for isolating known MRSA positive patients as well as for pre-emptive isolation. Though others report that the number of available side rooms in the UK is low (Cunningham R *et al*., 2007; Hardy K *et al*., 2010), if none are available, contact precautions can be applied to an individual who remains on the open ward. However, wherever the patients are placed, pre-emptive isolation will only function if good infection control practice such as compliance with hand hygiene is adhered to. A rapid screening test on admission, even if done at the point of care, will

take at least 70 minutes to produce a result, which is slower than the strategy of pre-emptive isolation described above. What is more, we can assume that the test will not be done immediately on arrival in the hospital, as other tasks will be prioritised. One operational study has reported the time to result of the Cepheid GeneXpert test as 7 hours and 50 minutes (Hombach M *et al*., 2010). Therefore rapid admission screening, at the point of care or otherwise, should be coupled with pre-emptive isolation. This will allow the resources required for the application of contact precautions such as side room use, and the donning of gowns and gloves to be optimised while interrupting the cross-transmission of MRSA.

7.2 Costs

False positive PCR tests and a low PPV of these tests mean that when implemented in operational settings, culture for MRSA needs to continue. However this is resource intensive and raises the cost. This may be necessary anyway if MRSA susceptibility tests are needed both to guide treatment of infection and decolonisation therapy. However a false positive PCR test will lead to unnecessary decolonisation, isolation and changes to peri-operative antibiotic prophylaxis, all of which incur their own financial cost and will mean further treatment for the patient. In this study, we waited for both negative culture and negative PCR admission screen results before removing patients from the pre-emptive isolation which was in place for MRSA control. However, the IDI-MRSA™ test had a high negative predictive value (99.4%) and the relaxation of this cautious approach would make IDI-MRSA™ screening much more cost-effective. One study estimated that the introduction of PCR screening for high risk patients reduced the number of pre-emptive isolation days for such patients by 60% at a cost of €95.77 (BD GeneOhm assay) or €125.43 (GeneXpert) per isolation day avoided (Wassenberg MWM *et al*., 2010). However, chromogenic media reduced the number of pre-emptive isolation days by 47% at a cost of €6.74 per isolation day avoided. These authors calculated the cost of an isolation day as €26.34, therefore only screening using chromogenic media was a cost saving procedure. An assessment of the effect of each method on MRSA acquisition rates was not performed.

The cost of PCR tests is affected by the automation of the testing. Though the amount of capital spent on the equipment is greater than for culture testing, the automation means that the process is more cost-efficient. However, with the IDI-MRSA™ test the extraction of the DNA is not automated and it is labour intensive. Further, the IDI-MRSA™ at the time of this study was validated for use on the Cepheid Smart Cycler. One Smart Cycler platform had the ability to process 14 specimens and two controls at one time during one cycle. It is possible to connect six Smart Cyclers in series so that 94 specimens and two controls can be processed during one cycle. However this comes at a price. The ability of mass processing to reduce the cost of the PCR tests has been raised and one group has validated a method, which is in use in their hospital **(**Paule SM *et al*., 2007). They compared the cost of mass processing using a method called ACP lysis in comparison to the validated IDI-MRSA™ method of single specimen processing. With the standard method they calculated that to process 14 specimens, 22 minutes of hands on time was required but the ACP lysis method need only 3 minutes. This resulted in a reduction of labour costs from US$11 to US$1.50. The time saving increased as the number of specimens processed increased; 14 samples saved 12 minutes and 98 samples saved >2hours. They report that a single technologist can process 120 – 150 tests in an 8 hour shift adding US$0.36 per specimen for extra reagents. The sensitivity, specificity, PPV and NPV of the ACP lysis method were 98%, 98%, 75% and 99.7% during the initial evaluation and when this was introduced in to routine practice, the PPV and NPV were 73.5% and 99.9%. The prevalence was 6%. An initially high unresolved rate of 11% was reduced to <1% in practice. However, it is important to note that such automated mass-processing is only relevant to larger laboratories. Further, the FDA has classed PCR testing as of moderate to high complexity. This means additional cost for these tests. The 'high' grading usually refers to some of the fine pipetting that is required for the extraction of the DNA and the manufacturers of these tests have made attempts to address this. However, culture testing is classed as of moderate complexity and so the qualifications required for a worker to process specimens in this way are less and thus this individual is paid at a lower salary scale. What is more, culture, particularly when a broth step is used, allows multiple specimens from the same patient to be pooled which improves the cost benefit of culture testing in comparison to PCR. As previously mentioned, the PCR tests have not been licensed for use on specimens from anatomical sites other than the nose and have not been licensed for use on

pooled specimens. Indeed specimens from sites with a greater number of inhibitors such as the rectum or axilla may result in a greater number of unresolved results, which would reduce the likelihood of a rapid result and necessitate greater expenditure. Though some of the PCR systems are categorised as closed and do not require the physical separation of the DNA extraction from the DNA amplification phase, contamination is still possible and many prefer to separate the two arms of the process. This requires extra laboratory space. It is clear that a formal cost-benefit analysis of PCR testing in comparison to culture testing is needed.

7.3 Further Work

Eleven studies investigating the impact of PCR admission screening on MRSA transmission rates have been cited in the present thesis. The findings vary despite similar clinical settings. Several questions are raised by the differences but there is often a single, key factor, such as pre-emptive isolation in the present thesis, which stands out as the potential reason for these differences. As a result the individual components of MRSA control programmes need investigation in order to address some of those theories. Their relative roles need clarification. With the present thesis in mind, the roles of rapid testing and pre-emptive isolation need determination. The prevalence of MRSA has fallen, and possibly other organisms that require isolation, in part due to good infection control. Consequently policies such as pre-emptive isolation, which have been met with reservation by some in the field, need re-addressing. At present, rapid tests refer to point of care tests and those based within the laboratory which may be PCR-based or chromogenic agar. Given some of the results found by others relating to cost-efficiency, each of these methods including chromogenic media should be given consideration. Within those studies, the tests themselves should be validated and the investigation of discrepancies carried out. The reasons for false positive and negative tests and the calculation of predictive values especially in the context of pre-emptive isolation of high-risk cases need further investigation. As we strive to determine the most effective and cost-efficient way to control MRSA, the costs of each strategy needs to be studied in a real-world setting, ideally in a multi-centre trial.

Chapter 8: Conclusions

Meticillin resistant *Staphylococcus aureus* is a significant cause of morbidity and mortality in the UK and other industrialised countries. Consequently, its control is an NHS and political priority and technological solutions have clear appeal. One recent innovation is the development of PCR based tests for the rapid detection of MRSA carriers, which would be expected to improve control.

The trial presented in this thesis is the only randomised controlled trial of rapid testing for MRSA. It set out to assess a rapid test for MRSA in a typical NHS setting. The PCR test performed well in both the validation stages and when in operational use. However in this study on general wards a rapid MRSA screening result did not, by itself, reduce MRSA rates to a degree likely to justify the cost. This hospital has MRSA pressure and infection control practices similar to or more intensive than comparable NHS institutions. In principle, the rapid detection of MRSA will have greatest effect where control measures are relatively weak for patients with undiagnosed MRSA carriage on admission. In the present study 30% of all patients subsequently found to be MRSA culture positive on admission were pre-emptively isolated prior to test results, but this is not universal practice elsewhere. Additionally, our policy is to nurse patients with standard precautions for their entire hospital stay, regardless of their MRSA risk factors. It is therefore possible that a more positive result would be seen for rapid testing in settings where these practices are employed only for those identified with MRSA. Rapid testing may have a role in outbreak control or in high-risk patients such as on ICU. In general medical and surgical settings prioritising rapid testing over optimising other control measures such as good infection control practice, compliance with high risk patient or universal MRSA screening and pre-emptive isolation of MRSA high-risk patients is not supported by the study presented in this thesis.

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Appendices

The pooling study paper and the clinical trial paper